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STRAWBERRY ROOT ROT IN RELATION TO MICROBIOLOGICAL CHANGES INDUCED IN ROOT ROT SOIL BY THE INCORPORATION OF CERTAIN COVER CROPS[1]

BY A. A. HILDEBRAND[2] AND P. M. WEST[3]

Abstract

Strawberry plants, variety Premier, were grown in naturally-infected root rot soil in which consecutive "crops" of several agricultural plants had been turned under, and in other lots of the same soil that had been steam sterilized or fertilized with barnyard manure. On examination of their roots it was found that the incidence and severity of root rot were closely correlated with soil treatment. Plants grown in sterilized soil remained free from disease as did those of the soybean series until the third season when they were slightly affected. Plants in the manure, corn, red clover, timothy, and untreated soil series all became diseased, the severity of attack increasing in the respective series in the order named.

Although roots of the various cover crops were found to contain representatives of several different genera of fungi, a specific fungus was dominant in each as was the nematode, *Pratylenchus pratensis*, in timothy and clover. This build-up of specific organisms *appeared* to be correlated with the incidence and severity of the disease in the roots of the strawberry plants that followed in the respective series.

However, in strawberry plants grown in the variously treated soils, fungal infection was negligible and, on the whole, not related to that of the preceding cover crop. An exception to this was the heavy infection by the mycorrhizal fungus (*Rhizophagus* sp.?), following timothy and corn, but a lack of correlation, in many cases, between the presence of the fungus and discoloured and necrotic tissue indicated that other agencies might be responsible for the injury. In timothy and red clover treated soils, nematodes, particularly *Pratylenchus pratensis*, might have been an important factor.

Fewer bacteria were found adjacent to roots of healthy plants than to those of diseased ones. Qualitative differentiation on the basis of nutritional requirements indicated a striking relationship between the incidence of certain groups of bacterial isolates and the severity of disease attack. The equilibrium between presumably "harmful" bacteria and the innocuous, normally occurring rhizosphere types is designated the Bacterial Balance Index. There are marked differences in the microbiological equilibria of the different soil series; increased severity of root rot is associated with a fall in the Bacterial Balance Index.

Introduction

Since the control of strawberry root rot obviously cannot be carried out under practical conditions by soil sterilization, and since satisfactory resistant varieties are not available, the most promising solution of the problem would appear to be a biological one. In a recent comprehensive review of the literature relating to soil-borne fungi and the control of root diseases, Garrett

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Co-operative project, Science Service, Department of Agriculture, Ottawa. Contribution No. 653 from the Division of Botany and Plant Pathology; contribution No. 118 (Journal Series) from the Division of Bacteriology and Dairy Research.

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(2) discusses very fully "the growing promise of biological control as a practical agricultural method" and cites a number of instances in which the method has been used with success in the control of certain soil-borne pathogens (8, 1, 10, 5). Because of such successes, the possibility of biological control as applied to strawberry root rot has been explored, efforts having been directed towards inactivation of suspected pathogens in the soil before the growing season. In this paper are presented results of experiments, carried out under greenhouse conditions, that show that incidence and severity of the disease can be modified by certain green-manurial treatments and that these modifications are correlated with changes in the microbiological equilibria of naturally infected root rot soil.

Description of Experiment

The soil used in the experiment, a light sandy loam, was obtained in the early fall of 1936 from a strawberry plantation which had failed that year because of high mortality of plants following attack by root rot. Soil from this heavily infected area after being thoroughly mixed was transferred to the greenhouse in 9-in. clay pots.

During the winters of 1936–37, 1937–38, and 1938–39, successive crops of tomatoes, corn, red clover, soybeans, oats, rye, timothy, and rape, respectively, as well as combinations of these plants in rotational schemes, were grown in different lots of the root rot soil. At the peak of their stage of succulence, the plants were chopped up finely and incorporated with the soil in which they had grown. In a winter season three or four crops of the faster growing plants like soybeans or tomatoes could be turned under but only one or two sowings of a slower growing plant like timothy in the same period of time. In a series like red clover–oats–corn, it was possible to complete the rotation only once during a winter. Additional series in the experiment included those in which well rotted manure and weeds instead of agricultural plants were added to the root rot soil. Two other series included untreated (control) root rot soil and the same soil sterilized with steam before each summer planting of strawberries. Altogether at the outset, the experiment included 16 different series with three pots to a series. At the end of the second summer, however, results indicated that nothing was to be gained by continuing nine of the series and they were discontinued. In this paper data are presented for the seven remaining series that showed most interesting and promising results, namely, timothy, soybeans, corn, manure, red clover, sterilized root rot soil, and untreated root rot soil.

In the summers of 1937, 1938, and 1939 the pots of soil were transferred from the greenhouse in the third week of August to outdoor plots of strawberries, variety Premier, where they were so placed that four vigorous runner plants could be struck in each pot. Each summer the pots were left outdoors until about October 1; at that time they were transferred to the greenhouse. There the strawberry plants were allowed to continue their growth until the third week in November, by which time they were approximately three

months old. They were then removed from the soil for examination and dry weight determination.

At certain stages of the experiment selected material from the roots of the strawberry plants grown in the soils subjected to the different treatments, and from those of the cover crop plants, were examined microscopically. In addition, chemical tests of the soil were made using the Morgan universal rapid soil testing system, pH determinations being made at the same time.*

In November, 1939, when the roots of the strawberry plants grown in the variously treated soils were showing the most marked differences, it became possible to broaden the scope of the investigations to include bacteriological studies. Bacterial isolates were obtained from the rhizospheres of the strawberry plants of certain series, following procedures previously outlined by Lochhead (7) and by Taylor and Lochhead (11). Qualitative analysis of the bacterial flora and determinations of bacteriological equilibria were made according to the methods of West and Lochhead (15).

Macroscopic Examination of Roots

Strawberry Plants

In November of each year after the strawberry plants had completed the three-month period of growth for the season, they were removed from the variously treated soils and after being thoroughly washed were spread out in water against a white background for macroscopic observation. Following this, material was selected for microscopic examination (second year) and finally dry weight determinations were made. Since each series comprised three pots with four strawberry plants in each, it was possible to make observations and comparisons recorded below on a basis of 12 plants per series per season. Dry weights of the plants for each year are presented in Table I;

TABLE I

DIFFERENCES IN WEIGHTS OF ROOTS OF STRAWBERRY PLANTS GROWN IN TREATED AND UNTREATED ROOT ROT SOIL

Series No.	Treatment of root rot soil	Dry weight of 12 roots, gm.			Aggregate weights
		1937	1938	1939	
14	Steam sterilized	(1)† 72.0	(2) 33.9	(3) 25.6	131.5
4	Soybeans	(4) 35.0	(8) 25.0	(12) 20.1	80.1
16	Barnyard manure	(1) 31.3	(2) 23.4	(3) 16.2	70.9
1	Corn	(4) 36.0	(7) 19.0	(10) 12.6	67.6
3	Red clover	(3) 37.0	(6) 15.6	(9) 4.8	57.4
7	Timothy	(2) 20.0	(4) 10.2	(6) 8.7	38.9
15	Untreated (control)	23.0	7.8	3.4	34.2

† *Figures in parentheses indicate number of times cover crops were turned under and number of repetitions of other treatments.*

* *Within the range of accuracy obtainable by this method no significant chemical changes were noted, except that the pH of the root rot soil, originally 7.0 to 6.8, was lowered to and remained at 5.8 for some time following the turning under of each crop of soybeans.*

some appreciation of the qualitative differences between roots of the various series, which are described below, may be gained from Plate I, Figs. 1 to 7.

Series 14. Steam Sterilized Soil

Plants grown in the root rot soil that was sterilized each year just before the runners were struck, consistently developed virtually flawless roots. The white, silky lustre of even the finer laterals, together with their highly turgid condition gave evidence not only that these roots were free from disease but also that, physiologically, they were still functioning normally. As reference to Table I will show, the aggregate weight of the roots of this series over the three-season period was 131.5 gm. This weight exceeds by 51.4 gm. that of the roots of the soybean series which ranked second best. It will be noted further that the weight of the plants in this series decreased in successive seasons. This decrease in root production cannot be attributed to an increasingly important disease factor because from the pathological standpoint the roots grown in 1939, which weighed only 25.6 gm., were just as healthy as those grown in 1937, which weighed 72 gm.

Series 4. Soybeans

From the standpoint of both bulk and health, the roots of strawberry plants of the soybean series approached most closely those grown in sterilized soil. In 1939, they showed some discoloration but were still markedly superior to roots of any of the remaining series.

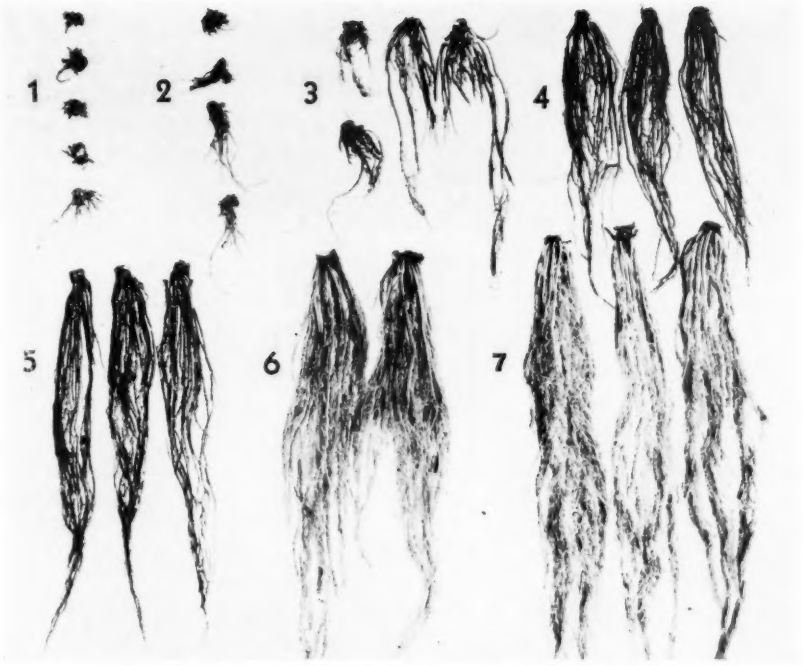
Series 16. Barnyard Manure

It will be noted in Table I that the aggregate dry weight of roots of the manure series was only 9.2 gm. less than that of roots of the soybean series. Despite this rather close parallelism in bulk of root production the plants of the manure series from the standpoint of health did not compare at all favourably with those of the soybean series. Whereas the latter showed little discoloration even in the third season, the former each year showed an almost general reddish-brown discoloration as well as a considerable number of definitely diseased main roots and laterals. The impression was gained from plants of this series, that during or following a period of apparently rapid and extensive root formation, some agency in the soil had brought about relatively quickly the condition of the roots described above. That such a condition in this or other series is to be distinguished from that accompanying or resulting from normal senescence would seem to be proved by the fact that roots of plants *of the same age* grown in sterilized soil showed no signs of ageing.

Series 1. Corn

In 1937, the roots of the strawberry plants grown in the soil after the incorporation of four crops of corn, were fairly good. In bulk they even slightly exceeded those of plants following the soybean series. However, in 1938 and 1939 their condition was about midway between that of plants of the best and poorest series. They showed general brownish discoloration and a relatively high proportion of dead and dying roots.

PLATE I



Roots of three-month-old Premier strawberry plants, 1939 series, showing correlation between incidence and severity of root rot and manurial and other treatments of naturally infected soil. FIG. 1. *Root rot soil untreated.* FIGS. 2, 3, 4, AND 6. *Root rot soil plus 9 red clover, 6 timothy, 10 corn, and 12 soybean cover crops, respectively.* FIG. 5. *Root rot soil plus 3 applications of barnyard manure.* FIG. 7. *Root rot soil sterilized with steam.*

Series 3. Red Clover

The results observed in this series proved to be very interesting and most surprising, especially when they are compared with those recorded for the soybean series. Since both are leguminous plants, it might reasonably be expected that their effects would be the same. The results obtained in 1937 suggested that such would be the case because that year the weight of the roots of the strawberry plants grown after the turning under of the first three crops of red clover slightly exceeded that of the plants of the soybean series. Pathologically there was little at the time to differentiate the roots of the two series. In 1938, however, the roots of the red clover series had dropped from second best in the scale to third poorest. Their discoloured and necrotic condition suggested an advanced stage of root rot. In 1939, the 12 strawberry runner plants struck in the soil with which nine crops of red clover had then been incorporated, developed so few roots that their total dry weight was only 4.8 gm. The few roots that had developed were without exception either badly discoloured or in an almost completely necrotic condition. In fact, it was difficult to find sufficient suitable material for microscopic examination.

Series 7. Timothy

Every year, roots of strawberry plants following timothy were very poor. In 1937, after only two crops of timothy had been turned under, the strawberry plants developed roots that were even poorer than those of plants grown in the untreated root rot soil. In 1939, three of the strawberry runner plants, like the 12 in the red clover series of the same year, produced almost no roots at all. The remaining nine runners developed to a slightly greater extent the semblance of a root system which at the time of the examination had reached an advanced stage of disintegration. It will be noted that the aggregate weight of the roots produced by all 36 plants of the timothy series over the three-year period was only 38.9 gm. These plants gave the impression that some powerful agency in the soil was not only inhibiting root development but was contributing to the destruction of the relatively few roots that were formed.

Series 15. Untreated (Control) Soil

In 1937, only the plants of the timothy series were poorer than those of the unsterilized soil or control series. By 1939, the soil had become so "sick" for strawberries that the survivors of the few roots that were formed weighed only 3.4 gm. The total weight of the roots produced by the 36 plants of this series for the three-year period was considerably less than half and only about one-quarter of that of the roots of the soybean and sterilized soil series, respectively.

Cover Crop Plants

In general, roots of the cover crop plants received only passing attention unless something unusual in their condition invited closer scrutiny. In corn no diminution of growth was noted in successive crops and the roots of

plants of the tenth sowing appeared to be as healthy as those of the first. In timothy and red clover, the sowings of the third winter season yielded less general bulk of plant than those of the first two years. The first eight sowings of soybeans resulted in excellent stands of plants, the roots of which never showed more than slight indications of discoloration and necrosis. However, the ninth sowing (i.e., the first of the third winter season) was almost a complete failure. A considerable number of the seeds germinated but gradually most of the young plants died, leaving finally only a few weak survivors. The roots of the latter were badly rotted and microscopic examination of necrotic tissue revealed extremely heavy infection by *Thielaviopsis basicola* (Berk.) Ferraris. It was thought at first that the fungus might have been introduced in or on the seed but, as attempts to culture it from a surplus of the latter proved unsuccessful, there seemed little doubt that the organism was already present in the soil. There is no reason for believing, however, that *T. basicola* was entirely responsible for the failure of this particular planting of soybeans. Rather, it would seem that some fault in the seed was a contributing factor. In three subsequent plantings, in which a new lot of seed was employed, excellent stands of plants were obtained, the roots of which though readily showing visible evidence of black root rot were, however, never affected severely enough to cause appreciable inhibition of growth of above-ground parts.

Microscopic Examination of Roots

In regard to the microscopic examination of the roots of the various plants in the experiment, one of two procedures could have been followed. Material for examination could have been selected either at periodic intervals throughout the experiment or at a comparatively late stage when it might reasonably be expected that an end point had been reached. Although the first mentioned procedure would have been highly desirable from the standpoint of detecting and following gradual microbiological changes, nevertheless, its adoption would have entailed the examination of a much greater amount of material than the second. As this would have required much more time than was available for this phase of the experiment, the second was adopted.

Roots of Cover Crop Plants

Material for examination was selected from roots of the cover crop plants of the first sowing of the third winter season, i.e., the fifth, seventh, eighth, and ninth crops of timothy, red clover, corn, and soybeans, respectively. Employing the technique that had been used with success in similar investigations (12, 3, 6) sufficient material was prepared to permit the examination of a minimum of 1000 mm. of root tissue of each cover crop. An attempt was made to select material that was not only comparable in age but which appeared to be in a seemingly similar pathological condition. Attention was confined primarily to rootlets showing the earlier stages of discoloration and necrosis since in such the chance of primary parasites being obscured by

TABLE II

INFECTION VALUE OF FUNGI AND INCIDENCE OF NEMATODES PER 1000 ML. OF ROOT TISSUE OF COVER CROP PLANTS

Series No.	Cover crop	Fungi observed and infection values assigned							Total infection value of fungi	Nematodes	
		Rhizoctonia Solani	Phycomycetous mycorrhizal fungus	*Asterocystis*	Unidentified fungi	*Thielaviopsis basicola*	*Pythium* spp.	*Helminthosporium*		Number	Kind
2	Tomatoes (5)*	794	79	31	61	—	—	—	965	48	Representatives of species more commonly regarded as saprophytes.
4	Soybeans (9)	—	Trace	24	Trace	2695	14	—	2733	73	
1	Corn (8)	—	307	43	583	—	1483	139	2555	70	
7	Timothy (5)	—	415	73	125	—	154	16	783	732	Mostly the parasitic species *Pratylenchus pratensis.*
3	Red clover (7)	—	1897	—	—	—	—	—	1897	960	

* *Figures in parentheses denote number of times cover crops have been turned under.*

TABLE III

INFECTION VALUE OF FUNGI AND INCIDENCE OF NEMATODES PER 1000 ML. OF ROOT TISSUE OF STRAWBERRY PLANTS GROWN IN TREATED AND UNTREATED ROOT ROT SOIL

Series No.	Treatment of root rot soil	Fungi observed and infection value assigned							Total infection value of fungi	Nematodes		Dry weight of roots, gm.
		Rhizoctonia		*Asterocystis*	*Alternaria*	*Pythium*	Phycomycetous mycorrhizal fungus	Unidentified fungi		Number	Kind	
		Orchid type	*Solani* type									
7	Timothy (4)*	3	Trace	—	—	—	2067	Trace	2070	536	*Pratylenchus pratensis*	10.2
1	Corn (7)	12	—	—	—	—	1617	—	1629	—	—	19.0
15	Untreated (control)	—	8	—	Trace	9	1341	Trace	1358	19	Saprophytic spp.	7.8
16	Barnyard manure (2)	—	—	—	—	—	828	—	828	—	—	23.4
4	Soybeans (8)	—	—	24	—	Trace	216	—	240	1	Saprophytic sp.	25.0
14	Steam sterilization (2)	Trace	—	—	—	—	—	—	—	—	—	33.9

* *Figures in parentheses indicate number of times cover crops turned under and repetitions of other treatments.*

secondary saprophytic invaders would be less than in rootlets in more advanced stages of disintegration. Starting at one extremity of a stained specimen, successive microscopic fields were examined and micro-organisms (chiefly fungi) observed in each field were assigned an empirical value based on the extent and degree to which the tissues were invaded. Summation of values for all microscopic fields in the examination of 1000 mm. of root tissue gave the total infection value for the fungal organisms observed. In the case of nematodes it was not necessary to assign infection values since for purposes of comparison the actual number of these organisms present in the tissues of roots of the different series could be counted accurately. In Table II are shown the infection values assigned the fungi and the numbers and kinds of nematodes observed in the roots of the respective cover crop plants.

Analysis of the data summarized in Table II reveals some interesting points. It will be noted that in each series, the infection value of one particular fungus alone exceeded that of the rest of the fungi combined. For example, in Series 1 (corn) the infection value of *Pythium* spp. was 1483, whereas the values of the mycorrhizal fungus, *Asterocystis*, unidentified fungi, and *Helminthosporium* combined amounted to only 1072. Similarly in Series 4 (soybean) the infection value of the predominating fungus, *Thielaviopsis basicola*, was 2695, and that of *Asterocystis* and *Pythium* combined was only 38.

A second significant point is the fact that it is not the same fungus that predominated in the different series. Thus, in the tomato series, the predominating fungus was *Rhizoctonia Solani*, in the soybean series, *Thielaviopsis basicola*, and in the corn series, *Pythium* spp. In both the timothy and red clover series, the infection value of the mycorrhizal fungus was highest. These results suggest that each of the cover crops has apparently favoured the development of a specific fungus, with consequent alterations in pre-existing microbiological equilibria.

In the case of nematodes, differences, both qualitative and quantitative, are no less striking than those recorded for the fungi. In the tomato, soybean, and corn series, a total of 191 nematodes was counted in the 3000 mm. of root tissue examined. Most of these represented species which are more usually regarded as saprophytic. In the timothy and red clover series a total of 1692 nematodes were counted in the 2000 mm. of root tissue examined. Almost without exception these were the meadow nematode, *Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936, which represents one of the most ubiquitous and important parasites of the roots of many plants.

Roots of Strawberry Plants

Rootlets of the strawberry plants that had completed the three-month period of growth of the second summer season were selected for examination. As in the case of roots of the cover crop plants care was taken to select comparable material. Infection values of the fungi and the numbers and kinds of nematodes were determined by examination of 1000 mm. of root tissue of straw-

berry plants following the respective cover crops. The data are presented in Table III.

Referring to this table, it will be noted that the infection values assigned the fungi vary widely, from zero for plants grown in the steam sterilized soil to 2070 for those grown in the timothy treated soil. In the soybean series, the fungi had total infection value of only 240, which, however, in the manure, untreated (control), corn, and timothy series increases to 828, 1358, 1629, and 2070, respectively. The series in which strawberries follow soybeans is one of the best to illustrate how an appreciable change has been effected in the original microbiological equilibrium of the root rot soil. It will be noted that the mycorrhizal fungus in the roots of the strawberry plants grown in untreated root rot soil has an infection value of 1341. In the roots of the strawberry plants grown in the same soil after the turning under of eight crops of soybeans, the infection value assigned the mycorrhizal fungus was only 216. Referring back to Table II, it will be noted that in the roots of the ninth crop of soybeans themselves, only trace infection by the mycorrhizal fungus was observed; *Thielaviopsis basicola*, however, was so abundant in the roots that it received the high infection value of 2695.

Unlike the fungi, nematodes do not gradually increase in number in the different series but are present in significant numbers in the roots of strawberry plants following timothy only. Almost without exception these belonged to the parasitic species, *Pratylenchus pratensis*.

The correlation pointed out above between soil treatment and degree of infection by fungi and nematodes is not the only one indicated by the data summarized in Table III. An apparent correlation exists also between growth response, as indicated by dry weight of roots of plants and infection by fungi and nematodes, particularly the former. In Series 14 (sterilized soil), where, except for traces of infection, fungi were absent, the roots weighed 33.9 gm., the maximum weight for all series in 1938. As the infection value increased to 240 in Series 4 (soybeans), there was not a proportional four-fold decrease in the weight of the roots. In fact, those of the manure series weighed only 1.6 gm. less than those of the soybean series. By referring back to the description of the macroscopic condition of the roots of these two series, it will be found that although in that season they did not differ appreciably in bulk, they did differ greatly pathologically; those of the manure series showed an almost general discoloration as well as considerable necrosis, whereas those of the soybean series had remained in an extremely healthy condition. In Series 1 (corn), where the infection value is 1629, the decrease in the weight of the roots was again quite marked. Such, too, was the case in Series 7 (timothy) and 15 (control), which make interesting comparison. In the former where the infection value of the fungi was highest, i.e., 2070, and where, too, presumably parasitic nematodes were most numerous, it might be expected that the dry weight of the roots would be lowest. However, the roots in this series weighed more than those of Series 15 (control) where the infection value of the fungi was only 1358. This suggests that in root

rot soil some factor or factors apart altogether from fungi and nematodes must be contributing to the almost complete inhibition in growth and destruction of strawberry roots.

Referring again to Table III it will be noted that the infection value of the phycomycetous mycorrhizal fungus so closely approximates the total infection value assigned all the fungi in each of the different series, that an attempt to evaluate the possible pathological significance of the fungi would resolve itself into giving primary consideration to this organism alone. Such consideration must still remain, however, mostly speculative since, up to the present, no one has succeeded in obtaining sufficient growth of the fungus on artificial media to be able to test its pathogenic capabilities. Consequently, in regard to its significance as a causal agent of root rot, little can be added to opinions already expressed by a number of investigators (9, 12, 3, 4). If the fungus is a parasite, then an interesting point disclosed by these studies is the ease with which its occurrence in root rot soil can be modified by different treatments—additions of corn and timothy apparently contributing to its increase, soybeans on the other hand sharply reducing its incidence. At this point mention should be made of the fact that in the roots of strawberry plants following soybeans, only scattered, incipient infections by this fungus were observed but in the roots of plants following corn and timothy, numerous heavily infected areas showed the fungus in all its stages. If the fungus is not a parasite, then the significance that seemed to attach to it in the correlations mentioned above must be attributed to some other factor or factors. Evidence in support of the latter possibility is not lacking. In many instances it is quite impossible to correlate the presence of the mycorrhizal fungus with discoloured or necrotic tissue. Nor is this true only of the mycorrhizal fungus. In the present studies and in others that have been carried out during the past few years*, some thousands of specimens of diseased roots have been critically examined. Rarely or never, have bacteria been observed *with certainty* in early infections, a fact which caused emphasis to be placed on the fungi in the search for the primary causal agent of the disease. In the majority of cases it has been possible to associate the presence of fungi (other than the mycorrhizal fungus) and nematodes with affected tissues. Cases were repeatedly encountered, however, in which no organism could be found in discoloured and necrotic tissue. In the aggregate the number of these cases reached such proportions that the phenomenon could not be regarded as being without significance. The impression was gained that many roots were injured not directly by actual invasion by parasitic organisms but indirectly by some agency in the soil. The exact nature of this agency remained unknown but circumstantial evidence over a long period of intensive investigation strongly suggested that it was more probably of biotic origin and could not be dissociated from the organisms suspected of being concentrated in root rot soil. The findings reported in the bacteriological section which follows are of considerable interest in this connection.

* *Results to be published shortly.*

Bacterial Equilibrium of Differently Treated Soils

As was pointed out above, the absence of organisms in discoloured and necrotic tissue of roots of many strawberry plants, strongly suggested the existence of a factor or factors capable of causing the type of injury in question without actual invasion of the roots themselves. Believing that bacteria might in some way be associated with these indirectly harmful effects, there was undertaken a qualitative survey of the general bacteriology of soils capable, on the one hand, of producing healthy plants and, on the other, of inducing symptoms of the disease. Five soils of the cover crop series, each differing significantly in its apparent content of the root rot producing principle were selected for bacteriological study. These were: Series 15, untreated root rot soil; Series 14, steam sterilized root rot soil; Series 16, root rot soil plus barnyard manure; Series 3, root rot soil plus nine crops of red clover; and Series 4, root rot soil plus 12 crops of soybeans.

It is obvious that the interaction between the general soil microflora and higher plants must occur at the root surface. Therefore, primary attention was paid to the isolates obtained from the rhizosphere of roots of the various series, though for purposes of comparison isolations were also made from soil beyond the influence of the roots. The technique employed has been described elsewhere (15, 7, 11).

Apart from any qualitative considerations, it is interesting to note the relative densities of the total bacterial populations that were found in the rhizospheres of plants manifesting varying degrees of root rot injury (Table IV).

Fewer bacteria were present in the rhizospheres of healthy roots than in those of plants affected with the disease. These differences occurred only at

TABLE IV

NUMBERS OF BACTERIA IN THE RHIZOSPHERES OF STRAWBERRY PLANTS IN RELATION TO SOIL TREATMENT AND DEGREE OF INJURY TO THE ROOTS

Series No.	Treatment of root rot soil	Rhizosphere bacteria, millions per gm. root	Bacteria apart from roots, millions per gm. soil	Relative severity of root injury**
15	Untreated (control)	2110	8	++++++++++
3	Red clover (9)*	1450	7	+++++++
16	Barnyard manure (3)	790	10	+++
4	Soybeans (12)	860	10	+
14	Steam sterilized (3)	250	28	—

* *Figures in parentheses indicate number of times cover crops were turned under or number of repetitions of other treatments.*

** *This approximation of injury to roots is based on macroscopic observation supplemented by comparison of dry weights for 1939.*

the root surfaces, the soils themselves (except that which was sterilized) supporting approximately equal numbers of bacteria; sterilized soil became repopulated to such an extent that more organisms were present in it than in soils of the other series.

The apparent correlation between bacterial activity at the root surfaces and the severity of injury by root rot indicated the necessity for qualitative analysis of the bacterial flora in each instance. To this end, the isolates from each series were differentiated into nutritional groups according to the method of West and Lochhead (15). Examination of the data thus obtained indicated a striking relationship between the incidence of certain groups of bacteria and the severity of attack by the disease (Table V). Bacteria of Group 3

TABLE V

PERCENTAGE OCCURRENCE OF VARIOUS GROUPS OF BACTERIA IN THE RHIZOSPHERES OF STRAWBERRY PLANTS SHOWING DIFFERENT DEGREES OF ROOT ROT INJURY

Bacterial group No.	Percentage occurrence				
1	0	10.9	0	10.6	18.6
2	29.3	2.2	15.4	0	4.6
3	**51.9**	**26.0**	**23.0**	**14.9**	**2.3**
4	0	2.2	0	10.6	0
6	1.3	2.2	0	4.2	2.3
8	6.6	8.7	0	2.1	4.6
10	5.3	23.9	38.4	12.8	20.9
5	**0**	**6.5**	**0**	**10.6**	**7.0**
7	**2.7** 5.4	**2.2** 23.9	**15.4** 23.1	**4.2** 44.5	**7.0** 46.4
9	**2.7**	**15.2**	**7.7**	**29.7**	**32.4**
Degree of injury	+++++++ ++++	++++ ++	+++	+	−
Series	Untreated soil	Red clover	Manure	Soybean	Sterilized soil

accounted for 51.9% of all organisms present at the root surface of the badly diseased plants in untreated root rot soil. These organisms became less and less abundant as the roots in the remaining four series showed greater and greater freedom from disease until, in sterilized soil, only 2.3% of the rhizosphere population was of this type. On the other hand, Groups 5, 7, and 9, which are known to be specifically favoured at the root surface of certain healthy plants (13, 14) comprised 46.4% of the bacteria in the rhizosphere of the healthiest roots, and only 5.4% in that of plants most severely affected with the disease. It is evident that the normally occurring rhizosphere types (Groups 5, 7, 9) which decrease in number as the disease becomes more severe, are at the same time being replaced by organisms of Group 3 (Fig. 1). This equilibrium has been conveniently expressed numerically as the "Bacterial Balance Index" (15). The remaining groups of bacteria were less well represented and do not appear to be correlated with the incidence of the disease.

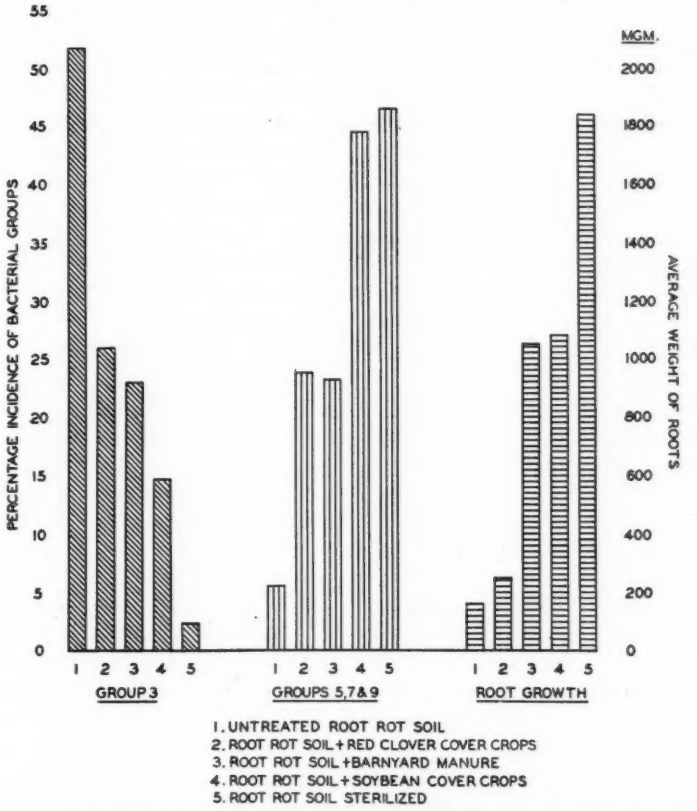


FIG. 1. *Correlation between occurrence of harmful (Group 3) and normally occurring rhizosphere types (Groups 5, 7, 9) of soil bacteria and severity of root rot as indicated by dry weights of surviving roots.*

The striking relationship existing between the kinds of bacteria occurring on strawberry roots and the severity of the disease is clearly shown by a comparison of the Bacterial Balance Indices of rhizosphere soils from each of the five series of plants (Table VI).

TABLE VI

THE "BACTERIAL BALANCE INDEX" OF SOIL ADJACENT TO AND APART FROM THE ROOT SURFACES OF STRAWBERRY PLANTS SHOWING DIFFERENT DEGREES OF ROOT ROT INJURY

Series No.	Treatment of root rot soil	Relative severity of the disease	Bacterial Balance Index	
			Rhizosphere soil	Soil apart from roots
15	Untreated	++++++++++++	−46	10
3	Red clover	+++++++	− 2	13
16	Barnyard manure	+++	0	19
4	Soybean	+	30	32
14	Steam sterilized	−	44	35

Corresponding values calculated from qualitative data for soils apart from the zone of influence of the root are also shown. It is apparent from these values that very marked differences exist in the microbiological equilibria of the five soils, and that the lower the Bacterial Balance Index falls, the more intensified the root rot factor becomes. The differences that occur between the five control soils (i.e., apart from the roots) are paralleled to an accentuated degree in the rhizospheres of plants grown in those soils. It is interesting to note that the incorporation of soybean cover crops into root rot soil causes a shift in the bacterial balance in the same direction as that produced by sterilization, the change being accompanied by a decrease in the root rot factor. The other leguminous crop, red clover, causes relatively little change in the equilibrium and brings about correspondingly little improvement in the condition of the roots.

As demonstrated previously (14, 15) certain plants, including strawberries, when growing in a "healthy" soil, exert a modifying influence on the soil microflora such that the bacteria requiring amino acids or growth substances are favoured, whereas other organisms are reduced in numbers. This is referred to as the "normal" or "positive rhizosphere effect", and corresponds to an increase in the Bacterial Balance Index of the rhizosphere soil over that of the surrounding unaffected soil. As shown in Table VI the healthy plants growing in sterilized soil exerted the usual positive rhizosphere effect. However, when the soil balance shifts in favour of the types particularly associated with root rot (Group 3), the normal effect of the plant on the microflora on its root surface is modified. At first as in the soybean treated soil, the rhizosphere influence is merely neutralized, and then in definitely "disease" soils, it swings strongly in the reverse direction. The reason for this reversal is not yet known. This abnormal change in which the unfavourable Group 3 bacteria are selectively stimulated at the root surface is referred to as the "negative rhizosphere effect". Apparently if Premier strawberry plants are grown in a soil with a low Bacterial Balance Index, the normal beneficial influence of the plant is overcome and the bacterial equilibrium at the root surface becomes even more unfavourable than throughout the rest of the soil.

Some indication of the manner in which Group 3 bacteria may exert a harmful effect on strawberry roots was obtained experimentally as follows. Selected portions of clean white roots washed in distilled water and cut into quarter-inch sections were placed in each of three Petri plates. To one lot was added tap water, to the second the mixed liquid cultures of approximately 50 organisms isolated from the rhizosphere of healthy plants growing in sterilized soil and to the third the mixed cultures of organisms isolated from badly rotted roots grown in the untreated root rot soil. After 24 hr. at room temperature the roots immersed in the bacterial suspension from diseased plants were becoming definitely discoloured, and by 48 hr. they were deep brown to black in colour and were reduced to a soft, flabby consistency. Even after six days, the root sections in the other bacterial suspension were clean and white with no more discoloration or loss of firmness than those in

tap water. No attempt had been made to exclude accidental air contaminants in this work. Further investigation showed that the deleterious effects of the bacterial isolates from root rot plants were the result of the activity of certain components of the Group 3 organisms. A series of preliminary infection tests indicated that such bacteria are capable of causing stunting and discoloration of the roots of strawberry seedlings. These observations together with the data showing correlation between numbers of Group 3 bacteria and the severity of attack by the disease would seem to indicate that the bacteriological balance of the soil is a factor that can by no means be eliminated from a consideration of the strawberry root rot complex.

Discussion

Garrett (2, p. 26) has recently pointed out that "the soil population is a dynamic biological equilibrium". The results of the present studies add emphasis to the truth of this statement. They have shown that populations of organisms including representatives of certain groups of fungi, nematodes, and bacteria, which have become established in a certain soil and which, as a result, have rendered that soil inimical to the healthy growth of varieties of the cultivated strawberry, are amenable to change. The new microbiological equilibria which have become established following the various treatments are still dynamic. In some cases, as for example in timothy and red clover treated root rot soil, the new components which are apparently dominant in the altered equilibria, still exert a deleterious effect on the strawberry, whereas in others, as in soybean treated soil, the effect is just as markedly beneficial.

In the present studies associative phenomena of the various types of organisms concerned have not been studied in sufficient detail to aid in a clearer understanding of the principles and the mechanism of associative effects. Thus, it is not known by what process the potentially parasitic phycomycetoid type of mycorrhizal fungus, the definitely parasitic nematode *Pratylenchus pratensis*, and the composite group of "harmful" bacteria, practically disappear from soybean treated root rot soil, whereas in the same soil under similar treatment *Thielaviopsis basicola* so greatly increases. Whether in this instance, such changes are due to competition and antagonism alone, or whether they are related to a changing chemistry of the soil, is not known at the present time.

So far as known detailed qualitative studies of the rhizosphere flora of strawberry roots have not been reported heretofore. Such studies in the present investigations have shown that the incidence of certain groups of bacteria are definitely correlated with the intensity of the root rot factor. Thus it becomes imperative to broaden the concept of the microbiological factor, in relation to strawberry root rot. Heretofore, emphasis has been placed almost exclusively on the fungous component. Although little doubt exists as to the parasitic capabilities of certain fungi and nematodes, never-

theless the clear-cut bacteriological correlations observed indicate that certain bacteria must be equally important components of the microbiological complex. The extremely high concentration of "abnormal" types of bacteria at the root surface of diseased plants suggests that they may well be the primary cause of much discoloration that occurs on the diseased roots, as well as contribute to the general susceptibility of the plant to fungous attack.

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THE MICROBIOLOGICAL BALANCE OF STRAWBERRY ROOT ROT SOIL AS RELATED TO THE RHIZOSPHERE AND DECOMPOSITION EFFECTS OF CERTAIN COVER CROPS[1]

By P. M. West[2] and A. A. Hildebrand[3]

Abstract

Soybean and red clover, grown as cover crops and incorporated into strawberry root rot soil, showed a marked difference in ability to control the disease on variety Premier. Soybean caused a striking reduction in the incidence of root rot and a drastic shift in the bacterial equilibrium of the soil. Red clover had little effect on the severity of the disease or the general microflora of the soil.

A study of "rhizosphere effects" reveals that the characteristic differences between the *resultant* bacterial equilibrium of the soils in which the two leguminous plants were grown, could not be attributed to influences exerted by the latter in the living state. However, the bacterial types favoured *during decomposition* in experimental cultures of tissues of red clover and of soybean, each inoculated with root rot soil, were identical with those isolated from root rot soil with which red clover and soybean, respectively, had been incorporated. In contrast to the putrefactive decomposition of red clover, soybeans apparently underwent a carbohydrate breakdown that could be reproduced essentially in culture by the substitution of glucose for soybean tissues. Beneficial changes in the bacteriology of actual root rot soils could be induced by the decomposition of pure carbohydrate in place of soybean. The favourable alteration in the bacterial equilibrium was accompanied by a corresponding modification of the fungous flora such that potentially pathogenic forms were replaced by presumably innocuous ones. These carbohydrate treated soils were capable of producing strawberry plants with well developed healthy root systems. The ability of soybean to control strawberry root rot therefore seems to depend primarily on a carbohydrate type of breakdown in diseased soil, causing a highly favourable shift in the microbiological equilibrium. The decomposition of red clover, on the other hand, did not under the same conditions induce these salutary effects.

Introduction

Previously reported investigations by the authors of the present paper (7), concerning the possibility of biological control of root rot of strawberry, showed that under experimental conditions the severity of the disease could be reduced by the repeated turning under of certain cover crops. Soybeans were found capable of exerting a corrective influence second only to that following sterilization by steam. Incorporation of this plant into naturally infected root rot soil resulted in a striking reduction in the incidence of the disease, this highly salutary effect being accompanied by a marked alteration in the bacterial equilibrium of the soil. Red clover, on the other hand, exerted the least beneficial effect of all the cover crops tried and induced only minor qualitative changes in the population of the root rot soil. Because of the striking contrast in the influence of these two leguminous plants, they were selected for further study which, it was hoped, might suggest fundamental reasons underlying the difference in effectiveness of the various cover crops in modifying the severity of the disease.

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Experimental

The Influence of the Living Plant

It is apparent that the cover crop plants under consideration might exert their characteristic effects on the microbiological population of the root rot soil either while in the living state, or while decomposing in the soil, or both. Since it is well known that growing plants are capable of modifying the bacterial flora in the immediate vicinity of the roots (9, 10, 13, 14, 15, 19, 25), this phase of the problem was considered first. Soybean and red clover were grown from seed in pots of the highly "potent" root rot soil used in earlier investigations (7), and after six weeks, representative bacteria were isolated from the rhizospheres by methods previously outlined (7). The isolates were studied qualitatively by the procedure of West and Lochhead (26) and the bacterial equilibrium existing at the root surfaces of the two leguminous plants was expressed as the Bacterial Balance Index. A summary of these data together with corresponding figures for strawberry seedlings of Premier parentage grown for four weeks in the same soil are shown in Table I.

TABLE I

THE BACTERIAL BALANCE IN THE RHIZOSPHERES OF SOYBEAN, RED CLOVER, AND STRAWBERRY SEEDLINGS GROWN IN ROOT ROT SOIL

Soil	Percentage incidence of bacterial groups		Bacterial Balance Index	Rhizosphere effect
	Group 3	Groups 5, 7, 9		
Root rot soil control	7	17	+10	—
Strawberry* rhizosphere	48	20	−28	Negative
Soybean rhizosphere	42	15	−27	Negative
Red clover rhizosphere	13	32	+19	Positive

**Plants grown from seed originating from fruit of Premier variety.*

In conformity with earlier findings (7), the strawberry seedlings became severely affected with root rot and exerted a "negative rhizosphere effect". Although the occurrence of favourable bacteria (Groups 5, 7, and 9) in the rhizospheres of these plants was not appreciably altered, the unfavourable types (Group 3) characteristically associated with the root rot condition were tremendously increased, thereby causing a sharp fall in the Bacterial Balance Index. Contrary to expectations, the bacterial equilibrium in the rhizosphere of the healthy soybean roots was almost identical with that of the roots of the strawberry seedlings. In the rhizosphere of red clover, however, the bacteria requiring specific nutritive substances (24, 25, 26) were selectively stimulated, and a typical normal or positive rhizosphere effect was the result.

The final outcome of the soybean treatment of root rot soil is a pronounced increase in the Bacterial Balance Index of the soil in general, caused by the

reduction in numbers of unfavourable bacteria (Group 3) and an increase in the types normally associated with freedom from disease (Groups 5, 7, and 9). It is clear that if the influence of the living plant, i.e., the rhizosphere effect, were the important factor concerned in shifting the bacterial balance of the root rot soil, then red clover would cause a rise in the Bacterial Balance Index whereas soybean would exert no such beneficial effect. Since the reverse is actually the case, the conclusion must be drawn that the influence of the growing plant on the bacterial balance of the soil is a relatively minor one and does not determine the nature of the extensive bacteriological changes that occur when the legumes are grown and incorporated into root rot soil. Apparently more potent and significant effects are exerted by these cover crops in some other way.

The Influence of the Decomposing Plant

When it became apparent that the difference in the abilities of the two leguminous cover crops to alter the microbiological balance of root rot soils was not associated with the actual growth of the plants, attention was turned to a study of the bacteriological aspects of their decomposition. Leaves and stems of clover and soybean grown under identical conditions in root rot soil for six weeks were finely chopped, 2.5 gm. of each placed in separate six-ounce culture bottles, 50 ml. of tap water added, and the mixture inoculated with 0.5 gm. of root rot soil, the Bacterial Balance Index of which was known. After two weeks' incubation at 28° C., the decomposing tissues exhibited the characteristics summarized in Table II.

TABLE II

GENERAL CHARACTERISTICS OF DECOMPOSITION OF SOYBEAN AND RED CLOVER AFTER 14 DAYS

Plant	pH	Millions of bacteria per ml.	Odour	Characteristics of breakdown
Soybean	4.8	1.3	Clean, acid, aromatic	Retention of green colour
Red clover	6.6	147.0	Foul, putrefactive	Colour change to dark brown—disintegration almost complete

It is evident that the breakdown of the two leguminous plants under identical conditions was strikingly different. The sharp contrast between bacterial numbers alone is suggestive of qualitative variations in the two. Apparently the soybean underwent a predominantly carbohydrate fermentation with the production of volatile organic acids, whereas the decomposition of red clover was chiefly putrefactive.

Isolations were made from the decomposing plant tissues by plating on soil-extract agar (10, 17) and representative organisms from each culture

were studied qualitatively to determine what bacterial types added in the original inoculum were favoured during the decomposition of the two crops. The results are summarized in Table III. For purposes of comparison, other data are included to show the changes in the bacterial equilibrium that followed incorporation of the two cover crops into root rot soil (7).

TABLE III

MODIFICATION OF THE BACTERIAL EQUILIBRIUM BY DECOMPOSITION OF RED CLOVER AND SOYBEAN IN ROOT ROT SOIL AND IN ARTIFICIAL CULTURE

—	Percentage incidence of bacterial groups		Bacterial Balance Index
	Group 3	Groups 5, 7, 9	
Untreated root rot soil	7	17	+10
Root rot soil following incorporation of nine crops of red clover	12	25	+13
Decomposing red clover culture	23	30	+ 7
Root rot soil following incorporation of 12 crops of soybeans	3	38	+35
Decomposing soybean culture	0	42	+42

It will be observed that after several crops of red clover were added to root rot soil, there was a small increase in both favourable and unfavourable bacterial groups, resulting in little net change either in the Bacterial Balance Index or in the improvement of the soil for strawberries. An exactly parallel but more pronounced effect on the bacterial population was found to result from the decomposition of red clover tissues when inoculated with root rot soil.

The incorporation of soybean cover crops into root rot soil resulted in an inhibition of unfavourable bacteria and a pronounced stimulation of favourable types, accompanied by a striking improvement in the Bacterial Balance Index and in the productivity of the soil. Again, an exactly parallel and somewhat intensified effect on the bacterial equilibrium was found to be characteristic of the decomposition of soybean tissues when inoculated with root rot soil.

These data suggest that the difference in ability of soybean and red clover cover crops to alter the microbiological balance of root rot soils depends primarily on differences in the chemical constitution of the two plants. When the plants are added to the soil these differences are reflected in the nature of their decomposition—carbohydrate fermentation in the one and putrefaction in the other. The bacteria concerned in the former process are the types associated with "healthy" strawberry soils, those concerned in the putrefactive breakdown are not. Thus the incorporation of soybean cover

crops into root rot soils causes a very favourable shift in the bacterial equilibrium, whereas red clover lacks this capacity. The situation is complicated by the fact that variations occur in the chemical composition of a crop depending on its stage of growth (8, 11, 18, 21, 22, 23) and a green manure might therefore be expected to exert a greater influence on the microbiological equilibrium of the soil at one stage than at another. However, previous results (7) as well as those described above, indicate that between species of plants of the same age, grown under identical conditions, there are inherent differences in composition which are highly significant in determining the type of micro-organisms concerned in their breakdown in the soil.

The above findings suggested that if the stimulating effect of decomposing soybean on the development of favourable bacteria depends on the carbohydrate type of decomposition which the tissues undergo in root rot soil, then similar changes might be induced by the substitution of a pure carbohydrate for the plant itself. With this in mind, an amount of commercial glucose equal to the weight of soybean tissue previously used, was placed in a culture bottle, 0.1% yeast extract was added to provide the necessary growth substances for the favourable bacteria, and water and root rot soil were then added as before. After two weeks' incubation the culture emitted a clean, acid aroma and its pH was 4.3. Superficially at least the breakdown of soybean was very similar to the fermentation of glucose.

A qualitative study of representative organisms isolated from the culture revealed the close parallelism existing between the decomposition of soybean tissue and the fermentation of glucose. In the latter the proportion of unfavourable organisms was reduced to 3%, while numbers of favourable bacteria rose to 65%. Thus the breakdown of pure carbohydrate raised the Bacterial Balance Index to +62, even higher than did the decomposing plant itself (cf. Table III). The successful replacement of soybean tissues with glucose in these decomposition experiments was of especial interest, because there was suggested the possibility of a simple means of experimentally altering the microbiological equilibrium of diseased soils and of studying the effects of such changes on the incidence and severity of strawberry root rot.

The Significance of Carbohydrate Breakdown

The fact that a highly favourable shift in the bacterial equilibrium of strawberry root rot soil could be brought about in culture flasks by the decomposition of either soybean or glucose, suggested treating actual root rot soil with a carbohydrate to determine whether or not the root rot factor could be controlled as effectively by this means as by the incorporation of soybean cover crops. The highly "potent" root rot soil used in earlier investigations (7) was selected for trial. To this soil, commercial glucose was added at the rate of 25 gm. per 3-in. pot of soil. Another source of organic carbon (acetic acid) which was considered likely to undergo decomposition similar to that of glucose was added to other 3-in. pots of root rot soil until the pH of the latter reached 4.5. Care was taken to add only

sufficient acetic acid to provide a satisfactory substrate for bacterial activity but not enough to exert any sterilizing effect. Both treated soils and untreated control root rot soil were kept in a moistened condition at room temperature for four weeks. During this time a vigorous carbohydrate fermentation took place in the glucose treated soil, as evidenced by a definite aroma of volatile organic acids and a drop in pH from 6.8 to 6.2 after six days. After four weeks, the reaction of both treated soils had returned to the original pH of 6.8, indicating that oxidation of all the organic acids had occurred. At that stage, four healthy strawberry seedlings (Premier parentage) of uniform size, were planted in the soils treated with glucose and acetic acid and in the untreated soil. The seedlings were allowed to grow in the three soils for 30 days. As had been observed on other occasions, seedlings grown in the untreated soil made little progress—rather, it was found that they had retrogressed and their roots, originally white and healthy, had become almost completely discoloured and necrotic. In marked contrast, the seedlings in the treated soils grew very rapidly, developing into vigorous young plants, with healthy and extensive root systems. The weights of the roots of the four seedlings from each series are shown in Table IV.

TABLE IV

GROWTH OF STRAWBERRY SEEDLINGS IN ROOT ROT SOIL AND IN THE SAME SOIL AFTER DECOMPOSITION OF GLUCOSE AND ACETIC ACID

Soil treatment	Weight of roots of four seedlings, mg.
Root rot soil untreated	50
Root rot soil + glucose	395
Root rot soil + acetic acid	510

Ten weeks from the date of the original treatments, Premier runners were struck in the non-treated and in the glucose treated soils in which the seedlings had already been grown. At the end of 30 days the plants in the non-treated soil were noticeably stunted and their roots not only lacked bulk but showed numerous, extensive reddish-brown to black lesions. On the other hand, plants grown in glucose treated soil made rapid development and their roots were bulky, white, and healthy (Plate I). These findings made it evident that not only could carbohydrate breakdown completely and effectually replace that of soybean in shifting the bacterial flora in soil cultures, but could also induce, in presumably the same manner, all the microbiological changes attendant upon the conversion of a "potent" root rot soil into a decidedly more healthy one. Moreover, the beneficial effect on the runner plants was in no way impaired by the growth of the seedlings that preceded them, thus indicating a residual effect of the glucose treatment.

PLATE I

Roots of Premier runner plants grown for 30 days in untreated (left) and glucose treated (right) root rot soil, showing how effectively the decomposition of the carbohydrate has modified the severity of the disease.

Qualitative study of the bacterial flora in the three soils after the removal of the seedlings yielded the results shown in Table V. These make interesting comparison with those shown in Table III. The bacterial equilibrium of the root rot soil to which either glucose or acetic acid was added, shifted in the same way but to an even greater degree than soil that received soybean cover crops. Group 3 bacteria were reduced in numbers, whereas the incidence of Groups 5, 7, and 9 was enormously increased, causing a decidedly favourable upswing in the Bacterial Balance Index. Since carbohydrate fermentation appears to be the fundamental basis for these changes, it is only to be expected that the pure compounds would have a greater effect than the soybean tissues themselves. It will also be noted that the change in the bacterial equilibrium that occurred during the decomposition of both soybean and glucose in artificial culture was more pronounced than in soil; this apparently was due to the greater buffering effect in the soil.

TABLE V

COMPARISON OF THE BREAKDOWN OF SOYBEAN TISSUES WITH THAT OF GLUCOSE AND ACETIC ACID IN RELATION TO THE BACTERIAL BALANCE OF STRAWBERRY ROOT ROT SOILS

Soil treatment	Percentage incidence of bacterial groups		Bacterial Balance Index
	Group 3	Groups 5, 7, 9	
Root rot soil untreated	7	17	+10
Root rot soil after decomposition of soybean cover crops	3	38	+35
Root rot soil after decomposition of glucose	0	45	+45
Root rot soil after decomposition of acetic acid	3	47	+44

Since all micro-organisms of the soil are biologically inter-related, it might reasonably be expected that environmental changes that are great enough to alter materially the bacterial equilibrium, probably at the same time would be capable of exerting equally profound influences on the fungous flora. To determine the effect of the glucose and acetic acid treatments of root rot soil on the fungi, a rhizosphere study of the seedlings from the three series was made. After the roots were vigorously shaken in sterile water to remove bacteria for qualitative analysis, they were further washed in running water for 1 hr. From each series, 51 representative sections of root, about one quarter inch in length, were "planted" on the surface of acidified and non-acidified potato dextrose agar plates, three per plate. The development of fungi from these platings was followed microscopically for several days, identification being made either directly from the plates or from cultured hyphal tips. As a result of the thorough washing, the fungi obtained from the rootlets represented either those from within the root tissues or those in intimate contact with the root surface.

The various fungi obtained from the obviously diseased roots of the Premier seedlings grown in the untreated soil included representatives of genera that are almost invariably isolated from plants affected with root rot, and to which also different investigators have attributed pathogenic capability (Table VI). On the other hand the relatively healthy roots of the seedlings grown in soil in which glucose or acetic acid had decomposed, gave a quite different picture. Very striking is the reduction of *Cylindrocarpon* from 29.1% of all fungi at the root surface of plants in untreated soil,

TABLE VI

THE IDENTITY AND PERCENTAGE OCCURRENCE OF FUNGI ON ROOTS OF STRAWBERRY SEEDLINGS AS INFLUENCED BY THE DECOMPOSITION OF GLUCOSE OR ACETIC ACID IN ROOT ROT SOIL

Root rot soil untreated			Root rot soil + glucose			Root rot soil + acetic acid		
Fungi			Fungi			Fungi		
Species	Total number	%	Species	Total number	%	Species	Total number	%
Actinomycetes	1	1.8	Actinomycetes	6	16.6	Actinomycetes	2	3.9
Cephalosporium	1	1.8	Fungus A	1	2.7	Fungus C	1	1.9
Cylindrocarpon spp.	16	29.1	Fungus B	1	2.7	Fungus D	1	1.9
Fusarium spp.	24	43.6	*Fusarium* spp.	9	25.0	*Fusarium* spp.	13	25.5
Gliocladium	1	1.8	*Hormodendrum*	2	5.5	*Mucor*	4	7.8
Orchid *Rhizoctonia*	1	1.8	*Mucor*	2	5.5	*Penicillium* spp.	26	51.0
Penicillium spp.	6	10.9	*Penicillium* spp.	6	16.6	*Trichoderma*	4	7.8
Phoma	1	1.8	*Phoma*	1	2.7			
Trichoderma	3	5.5	*Trichoderma*	1	2.7			
Verticillium	1	1.8	*Verticillium*	1	2.7			
			Yeast	6	16.6			
	55			36			51	

to zero in the other two root systems (Fig. 1). Berkeley and Lauder-Thomson (2) and Richards and McKay (12) have ascribed importance to *Cylindrocarpon* species as primary parasites capable of inducing a characteristic type of root rot. In addition, the closely allied genus *Fusarium*, also of considerable pathological significance (2, 12, 20) was reduced by the carbohydrate treatment from 43.6 to 25.0%. The endophytic *Rhizoctonia* of the type found commonly in the Orchidaceae and frequently associated with root rot of strawberry (4, 6, 20) was isolated from the seedlings grown in the untreated soil but not from those in the treated soil. The reduction in percentage occurrence of the above mentioned fungi, or, in some cases, their complete disappearance, was necessarily accompanied by an increase of other types. The latter for the most part were considered to be "harmless" forms. The incidence of *Penicillium*, for example, increased from 10.9% in the untreated soil to 51.0% in the acetic acid treated soil. Others occurring more abundantly or exclusively in the treated soils included actinomycetes, yeasts, *Mucor*, and *Hormodendrum*.

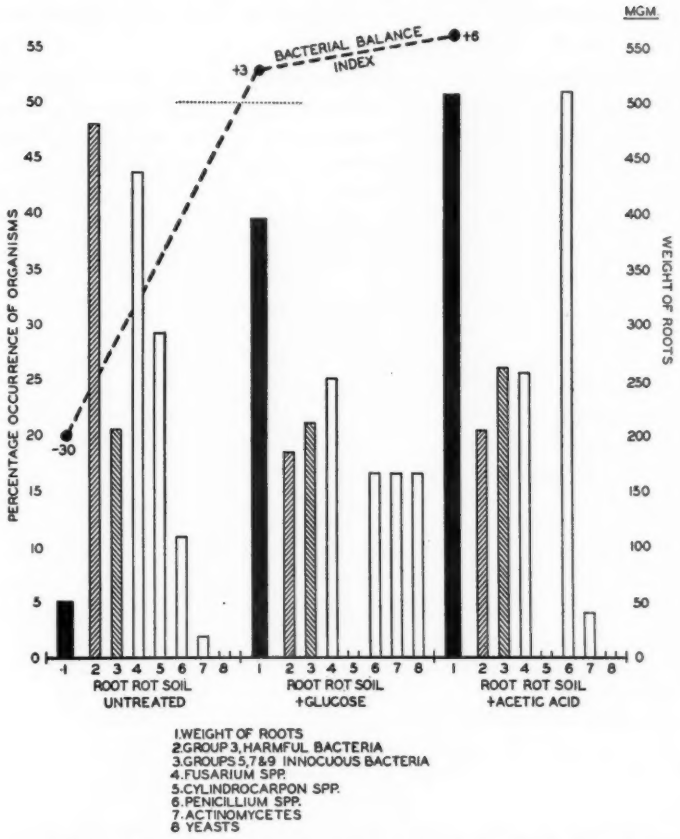

FIG. 1. *Influence of carbohydrate decomposition in root rot soil on the bacterial and fungal populations in the rhizospheres of Premier seedlings.*

In addition to the qualitative differences pointed out above, it will be noted (Table VI) that there are also quantitative differences. For example, whereas a total of 55 fungous isolates was obtained from the roots of the seedlings grown in the untreated soil, only 36 developed from those grown in the glucose treated soil.

The changes in the bacterial equilibria of the rhizospheres following carbohydrate treatment are similar to those already described for the soil apart from the roots. The Bacterial Balance Index was significantly increased, owing primarily to a marked reduction of Group 3 unfavourable bacteria (Fig. 1). Total bacterial counts in the rhizosphere were not widely divergent (248, 223, and 407 millions per gm. of root system for untreated, acetic acid, and glucose treated soils, respectively) in contrast with the differences in

density of population of the soil apart from the roots (11,100 and 370 millions per gm.).

The above results show, then, that the beneficial change in the bacteriology of root rot soil that followed the breakdown of carbohydrate was accompanied by correspondingly favourable modification of the fungous flora, some of the inter-relationships being illustrated in Fig. 1. Apparently the ability of soybean to modify so effectually strawberry root rot depends primarily on the carbohydrate type of decomposition which the plant undergoes in diseased soil and which produces a highly favourable shift in the general microbiological balance.

Discussion

As more and more data in connection with strawberry root rot are accumulated, the more the complexity of the disease is realized. To date, its cause has been investigated more intensively from the microbiological than from other avenues of approach. The microbiological factor, alone, is a most complicated one as the following brief consideration of a few of the papers that have appeared on the subject during the past few years will show. In 1931, Strong and Strong (16), in demonstrating in pot experiments the etiological relationship of the imperfect stage of *two* Ascomycetes, proved the infectious nature of the disease for the first time. In 1934, Berkeley and Lauder-Thomson (2) demonstrated, also in pot experiments that *five* different fungi (two of them probably identical with those of Strong and Strong) were capable of parasitizing strawberry roots and of producing root rots the symptoms of which were "very much alike, if not identical". Also, in 1934, Truscott (20) found that representatives of *seven* different genera of fungi possess parasitic capability. In the same year, Hildebrand (4) mentioned that nematodes had been encountered in association with diseased strawberry roots in numbers and frequency sufficient to suggest a possible causal relationship to the disease. Recently, the present authors (7) have demonstrated that in addition to a fungous factor and other biotic factors that may be involved, there is also a bacterial factor which can by no means be eliminated from a careful consideration of the strawberry root rot complex.

In any given soil these various organisms are in equilibrium with their environments and with each other and thus no single component of this inter-related system may be disturbed without affecting the whole. This point is illustrated by the decomposition studies described in the present paper, which demonstrate not only the ease with which the microbiological balance may be shifted but also the interaction that occurs between two important groups of components of the complex. Thus, in comparing the bacteriological and mycological data (Fig. 1), it becomes clear that the beneficial effects of the carbohydrate type of organic decomposition in soil as related to strawberry root rot, cannot be defined in terms of what happens to any one species of fungus or any one group of bacteria. Rather, it seems that it is the sum of all the microbiological changes that must be taken into

account in describing the influence of the soil treatments on the root rot picture.

An interesting observation concerning the corrective effect of carbohydrate decomposition on the microflora of root rot soils is the beneficial nature of the qualitative changes in both bacteria and fungi. The bacterial balance was altered in such a way that those organisms consistently associated with strawberry root rot were reduced, and harmless forms increased. Genera of potentially pathogenic fungi, which thrived together with unfavourable groups of bacteria, were replaced by other innocuous types at the same time as the bacterial equilibrium improved.

That these microbiological changes which occur as a result of carbohydrate breakdown in strawberry root rot soils may not necessarily prove beneficial in other root rots is suggested by the work of Eisenmenger (3). His observations indicated that carbohydrate decomposition increased the severity of brown root rot disease of tobacco. This may be correlated with the observations of Berkeley and Koch (1) who warned that previous crops of soybeans in certain types of soil might accentuate brown root rot of tobacco grown later. In the light of the concepts mentioned above, it would seem that the problem of control of the disease is probably not concerned with the elimination of any single organism but rather with effecting relatively profound changes in the general microbiology of root rot soil—such changes as are apparently induced by the incorporation of the soybean or certain carbohydrates as sources of energy. In general, it appears that the biological oxidation of carbohydrate materials in strawberry root rot soil reduces the severity of the disease by bringing about a marked modification of the bacterial and fungal floras of the soil in such a way that potentially "harmful" organisms are greatly reduced and a relatively more favourable microbiological balance becomes established.

Acknowledgments

The writers wish to express appreciation of the valuable assistance received from Dr. A. G. Lochhead, Dominion Agricultural Bacteriologist, Department of Agriculture, Ottawa, and from Dr. G. H. Berkeley, Officer-in-Charge, Dominion Laboratory of Plant Pathology, St. Catharines, Ont.

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EFFECTS OF PHYTOHORMONE, POTASSIUM NITRATE, AND ETHYL MERCURIC BROMIDE ON THE GERMINATION AND EARLY GROWTH OF WHEAT[1]

By N. H. Grace[2]

Abstract

Marquis wheat treated with a series of talc dusts containing indolylacetic acid, potassium naphthylacetate, potassium nitrate, and ethyl mercuric bromide was grown in soil in the greenhouse. Potassium naphthylacetate increased the final germination count 2.6% while indolylacetic treatments failed to affect germination or early growth. Neither of these growth stimulating chemicals interacted with potassium nitrate or ethyl mercuric bromide. Ethyl mercuric bromide retarded the germination rate but increased the final germination count. Potassium nitrate in conjunction with the organic mercurial disinfectant reduced final germination and the air-dry weight of young plants. On the average, dust treatments reduced the rate of germination but increased the air-dry weight of stems.

It has been shown that there are conditions under which phytohormone treatments of cereal and other seeds influence germination and early growth and even the final yields (1, 4, 6, 7, 9–11, 13, 15, 18–20, 23), though lack of effect, or injury from overdosage, have been noted more frequently (3, 4, 8, 10, 16, 17, 21, 22, 24, 25). Small amounts of certain nutrient salts applied to the seed in a dust disinfectant appeared to have some effects on early growth of wheat (12). In consequence, it was considered of interest to investigate the effects of combinations of phytohormone and nutrient salts with seed disinfectants on germination and early growth of wheat in the greenhouse. This communication describes the results of experiments in which wheat seed was treated with growth stimulating chemicals, potassium nitrate, and ethyl mercuric bromide in a talc carrier.

Experimental

The experiments involved treatments with dusts containing indolylacetic acid and the potassium salt of naphthylacetic acid. Each of these chemicals were used separately at concentrations of 0, 0.192, 0.576, 0.96, and 1.92 (parts of chemical in 100 parts of the talc mixture by weight) and in combination with 0 and 3.71% potassium nitrate and 0 and 5% ethyl mercuric bromide (5). These percentages of phytohormone chemical in talc when applied to seed at the rate of one-half ounce per bushel effected treatments of 0, 1, 3, 5, and 10 parts of chemical to a million parts of seed by weight. The entire series for each phytohormone chemical involved the preparation of 20 dusts. Master dusts were prepared first and by their mixture and the addition of talc, the various concentrations and combinations.

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[2] *Biochemist.*

Marquis wheat seed was treated with these dusts at the rate of one-half ounce of dust per bushel of seed in the manner previously described (12). Seed was planted, between 24 and 48 hr. after application of the dust, in small cardboard flats containing unsterilized soil, 50 seeds to each flat. The investigation involved two separate experiments. Experiment 1 comprised treatments with indolylacetic acid and Experiment 2, those with potassium naphthylacetate. Plants in the former were started February 15, 1940, and removed approximately six weeks later, whereas those of the latter were planted April 5 and the plants removed five weeks thereafter. Each experiment involved 10 randomized replicate blocks of the 20 dust treatments and, in addition, an untreated control flat to each block. Thus each experiment required 210 flats with a total of 10,500 seeds.

Temperature in the greenhouse room was maintained around 50° F. during the night and ranged between 55° and 60° F. in the daytime. Although temperature conditions were similar for the two experiments there was greater intensity and duration of the light for the second experiment owing to the advancing season.

Daily germination counts were made as soon as emergence commenced and the germination rate was computed by the method of Bartlett (2). The plants were washed to remove the soil and the air-dry weight of entire plants, as well as of stems and roots, was determined (11–13). All the data were subjected to analyses of variance.

Results

In Table I are given results of the analyses of variance of the data. Indolylacetic acid treatment failed to produce any significant effects whereas potassium naphthylacetate treatment affected the final germination count. Phytohormones failed to interact with the other chemicals. Organic mercury treatment had a marked effect on the germination rate in both experiments and on the final germination count in one. Potassium nitrate treatment interacted with organic mercury.

Experiment 1—Treatments with indolylacetic acid, potassium nitrate, and ethyl mercuric bromide

Organic mercury treatment effected a decrease of 2.4% in the germination rate, the value being 68.3% in its absence and 65.9% in its presence. Conversely, the final germination count was increased from 89.0 to 92.2%. The air-dry weight of plants was unaffected.

Experiment 2—Treatments with potassium naphthylacetate, potassium nitrate, and ethyl mercuric bromide

The growth stimulating chemical increased the final germination count, on the average, by 2.6%. In its absence the value was 88.4% and the average for its four concentrations, which did not differ significantly among themselves, was 91.0%.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF WHEAT SEED TREATED WITH TALC DUSTS CONTAINING PHYTOHORMONE, POTASSIUM NITRATE, AND AN ORGANIC MERCURIAL DISINFECTANT

Source of variance	Degrees of freedom	Mean square Experiment 1 (Indolylacetic acid)					Mean square Experiment 2 (Potassium naphthylacetate)				
		Germination rate	Final germination count	Air-dry weight of:			Germination rate	Final germination count	Air-dry weight of:		
				Whole plants	Stems	Roots			Whole plants	Stems	Roots
Replicates	9	0.1786***	31.93**	1.02**	0.680**	1.02**	0.0504***	8.84	0.624***	0.3097**	0.0586**
Treatments:											
Untreated versus treated	1	.0001	0.00	0.05	.003	0.05	.0164*	0.10	.047	.0661	.0018
Phytohormone	4	.0025	2.28	.54	.160	.16	.0011	19.39*	.025	.0077	.0046
Potassium nitrate	1	.0005	0.50	.76	.043	.30	.0003	13.52	.128	.0633**	.0123
Ethyl mercuric bromide	1	.0280***	118.58**	1.11	.249	.10	.0523***	21.78	.054	.0003	.0005
Interactions:											
Phytohormone × potassium nitrate	4	.0014	7.16	.66	.212	.26	.0017	11.83	.035	.0127	.0070
Phytohormone × ethyl mercuric bromide	4	.0024	5.34	.34	.062	.14	.0020	3.19	.050	.0193	.0018
Potassium nitrate × ethyl mercuric bromide	1	.0014	0.02	.51	.089	.13	.0104	27.38*	.197*	.1220**	.0059
Phytohormone × potassium nitrate × ethyl mercuric bromide	4	.0010	.43	.47	.178	.13	.0053	2.42	.014	.0134	.0022
Error	180	.0019	3.56	.32	.112	.12	.0030	6.42	.045	.0088	.0078

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

The average effect of organic mercury treatment was a reduction in germination rate per cent from 78 to 75.5. The average final germination was approximately 91%. Neither the mercurial nor potassium nitrate, applied separately, changed this, but, when these chemicals were used in combination, germination fell to 88%.

Neither organic mercury nor potassium nitrate treatment, alone, affected the air-dry weight of plants from 50 seeds, which averaged 2.70 gm. In combination these chemicals reduced the average weight to 2.61 gm. A similar interaction was demonstrated by the air-dry weight of stems from 50 seeds which averaged 1.90 gm. in the absence of both organic mercury and potassium nitrate. Combination of organic mercury and potassium nitrate resulted in a weight of 1.84 gm.

Dust treatments, on the average, reduced germination rate from 81%, for the untreated control, to 77%. Conversely, the air-dry weight of stems from 50 seeds was 1.80 gm. for the untreated, but averaged 1.89 gm. for the dust treated.

Phytohormone treatments, alone, or in combination with nutrient and disinfectant chemicals, failed to have any marked effect on germination or early growth of wheat under the conditions of these experiments. It seems probable that instances of stimulation from treatment of cereal seeds relate to the circumstances under which the observations were made.

It is of interest to note that the organic mercurial disinfectant tended to retard the rate of germination but increased the final germination count. Such increases have been noted in the literature (5). Whereas potassium nitrate treatment failed to show the stimulatory effect of the type noted in earlier experiments (12), it did interact unfavourably when used in conjunction with the organic mercurial. In contrast, similar combinations applied to plant stem cuttings have given favourable responses (14).

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THE INHERITANCE OF FRUIT SIZE IN THE TOMATO[1]

By L. Butler[2]

Abstract

It is pointed out that size data from over 50 tomato crosses are explained by the assumption of the geometric action of size factors but not by a simple additive theory.

The fact that the F_1 results fitted such a theory was pointed out in a previous paper when the theory was proposed. The analysis is here extended to the F_2 generation and to cell size measurements.

The use of the geometric scale introduces regularity into the otherwise unpredictable F_2 segregations, and they become amenable to a simple logarithmic scheme. Analysis by such a scheme indicates that differences in cell number or ovary size are caused by the segregation of three to five pairs of major genes, whereas mature cell size differences seem to be brought about by the segregation of at least twice as many factors.

Final weight is thus the resultant of the proportionate action of the following factors:—

1. The number of mitotic divisions in the pre-anthesis period and therefore the number of cells at anthesis.
2. The cell expansion after anthesis.
3. Fruit shape, locule number, and other size-modifying effects.

The science of genetics has been very severely criticized because it deals only with superficial characters and with freaks that are not essential for the continuance of the species; therefore it is gratifying to see the revival of interest in the inheritance of quantitative characters. Size is a highly complex and important character from the practical standpoint as well as from the pure science viewpoint. As it is the chief concern in the problem of hybrid vigour any rational rules that can be formed about its control and its determination will be of great assistance in solving this problem. Size is, moreover, one of the better known examples of multiple factor inheritance; since there is very little reliable information on this subject, the data presented here may help to solve some of the many problems connected with such a type of inheritance.

The tomato fruit, because of its wide range in size and the presence of marker genes in most of its chromosomes, presents excellent material for the study of size inheritance. The inheritance of fruit weight has been studied by Groth (10, 11, 12), who found that the F_1 weight was the geometric mean of the two parental weights. His proposed hypothesis of the "Golden Mean" in size inheritance was poorly received. Lindstrom (15), also working with fruit weight, found that the F_1 mean was much closer to the smaller parental value; he postulated the dominance of small size. The histological basis of differences in fruit size was investigated by Houghtaling (13) and Butler (2),

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both of whom arrived at the same conclusion by different methods of approach. They stated that prior to anthesis, since cell volume at that time was the same for all varieties, increase in size was by cell division so that ovary size is directly related to cell number. After anthesis growth is due chiefly to cell enlargement.

Recently MacArthur and Butler (17) as the result of extensive investigations of the inheritance of fruit size in the tomato have put forward a theory of inheritance based on geometric growth processes. By four different approaches they showed that inheritance of fruit size is of a proportionate or geometric nature and not the simple additive type which is usually postulated.

When parents with fruit of two different sizes were crossed the fruit size in the F_1 was close to the geometric mean of the two parental sizes. This was explained by the fact that the cell number of the fruits of parents and F_1 are in geometric progression, so that the number of cells in the F_1 is caused by an intermediate number of cell divisions. It was further intimated that inheritance of cell size seems to be of a somewhat similar type, but the data on cell size were not extensive. During these experiments further data were collected and found to substantiate these findings.

In this paper the analysis will be carried into the F_2.

The Segregation of Weight in the F_2

In their paper (p. 257), MacArthur and Butler give curves showing the F_2 frequency distribution of the fruit weights of two crosses in each of which approximately 1000 F_2 plants were grown. Examination of these curves shows that they are both strongly skew, g_1 being $+1.105$ and $+1.596$. Such a result is typical for the weight segregation in 56 crosses examined in this study. When the logarithms of the weights are plotted instead of the weights themselves, the curve becomes normal.

The regularity introduced into the measurements by using logarithms can also be illustrated in the following manner. Taking the two parental weights, the two extremes of F_2 range, and the F_2 mean the following five values are obtained:

P_1	F_2 Segregants			P_1
Small	Smallest	Mean	Largest	Large
1.1	2.3	7.6	25	56
1.2	5.3	17.4	31	

The second line is obtained by subtracting consecutive terms of the first row. The two inner values represent the spread of the range on each side of the mean. In a symmetrical distribution these two values would be the same, so that the departure from equality is an indicant of skewness. The two outer values show the distribution of the segregation with respect to the two parent values. If the inheritance was of the typical blending type these two values would be equal, but it is easy to see that the values are very unequal. The smallest F_2 fruit is almost 30 times closer in size to that of the smaller parent than the largest F_2 fruit is to that of the larger parent.

If the logarithms of these values are taken, the following series is obtained:

P_1	F_2 Segregants			P_1
Small	Smallest	Mean	Largest	Large
0.041	0.362	0.881	1.398	1.748
0.321	0.519	0.517	0.350	

It will be seen that the logarithms of the numbers fit into a definite series. The F_2 segregation occupies the central part of the parental range, the mean being 0.840 from the small parental weight and 0.867 from the large parental weight. The F_2 mean is now in the centre of the distribution instead of being closer to the smallest segregant.

The use of logarithms on the data for other crosses and the backcrosses produces the same type of regularity. Such behaviour leads to the assumption that the growth processes controlling fruit size act in a geometric manner, and with this in view, the segregation of these processes has been examined.

The Inheritance of Ovary Size in the F_2

It has been previously shown (2), because cell volume is the same in all varieties at anthesis, that ovary size is proportional to cell number. Therefore, because of its convenience, ovary size may be used as an indicator of cell number segregation in the F_2. The distribution of ovary sizes at anthesis, like that of fruit weights, is strongly skew in a positive direction but becomes normal when plotted on logarithmic paper. There is, however, one important difference which is readily apparent from an examination of Table I. In

TABLE I

SEGREGATION OF OVARY DIAMETERS

	Red Currant			Yellow Cherry Burbank Preserving	Yellow Peach		Pink Peach	902 selection	Devon Surprise		Tangerine
Ovary diameter	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
463 R.C. × Pk. Pch.	13	45	50	35	14	7					
3313 R.C. × 902 sel.	4	9	23	18	8	5	1	1			
3210 R.C. × Tang.	4	29	73	57	26	9	2				
3218 B.P. × 902 sel.			2	3	20	11	18	10	3		
3728 Y.C. × Tang.				1	15	40	33	14	6	3	2
522 Y.Pch. × Devon S.					9	51	59	23	4		

most of the crosses the parental values are recovered, indicating that ovary size is controlled by fewer factors than fruit weight. The parental fruit weights were not recovered even when 10,000 plants of the F_2 generation were grown. A second feature of these distributions is the strong positive skewness indicating either geometric action of the genes concerned or the dominance of small size. In an attempt to assess the probable number of

factors operating in these crosses, use is made of the binomial distribution. It is known that non-dominant genes of equal effect and equal frequency are distributed in classes according to the expansion of the binomial $(a + b)^n$. The number of genetic classes of different numerical value is, accordingly, $n + 1$ and the frequency of each class is given by the numerical value of the coefficient of each term in the expanded binomial. The number of pairs of allelomorphic genes is $n/2$ and the least number of individuals required to include all classes is 2^n.

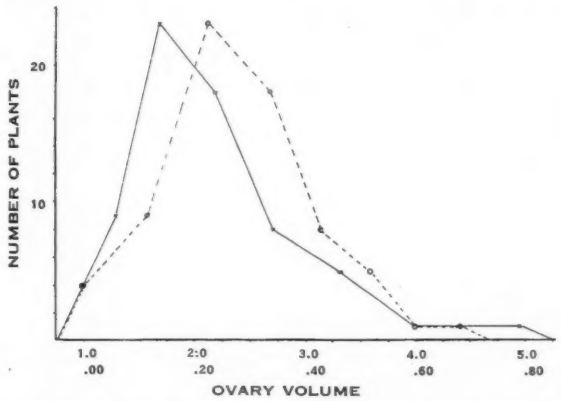

FIG. 1. *Distribution of ovary volumes in the F_2 of cross 3313 (Red Currant × 902 selection).* — x — *Arithmetic scale.* – – o – – *Logarithmic scale.*

Advantage may be taken of this last statement to estimate the number of genes controlling the difference in parental ovary sizes in cross 3313. Assuming that the large parent carries all the large-size producing genes and the small parent the less effective allelomorphs of these, then the extremes of range possible in this cross are the two parental classes. Then, since all classes were observed when only 69 individuals were measured, it is concluded that about three pairs of genes ($2^6 = 64$) are concerned here. Next the abscissa values or ovary sizes that correspond with the theoretical gene frequencies must be obtained. Now since gene action as far as ovary volume is concerned is geometric, a geometric or logarithmic scale must be used. Assuming as before that the two parental values are the upper and lower limits for this segregation, then the range of the logarithmic scale is $0.612 - \bar{1}.982 = 0.630$. To test the hypothesis of three pairs of genes, seven $(n + 1)$ equally spaced abscissal values are obtained by dividing the range by n and getting 0.105 as the interval between classes. Then starting from the value of the smaller parent and adding 0.105 each time the required class values given below are obtained.

Class value	$\bar{1}.982$	0.087	0.192	0.297	0.402	0.507	0.612
Observed frequency	1	5	12	23	15	9	3
Theoretical	1	6	16	21	16	6	1

Similar schemes may be worked out for the rest of the crosses. With the exception of those involving Tangerine, the theoretical and observed values in all crosses are similar. When Tangerine is one of the parents the distribution is biased towards the smaller parent. This bias may be explained by assuming multiple-allelomorphic action of some of the size genes.

The Inheritance of Mature Cell Size in the F_2

The size of cells of the locule wall is an inherent character of the variety, and the F_1 data indicated that a geometric type of gene action was responsible for its inheritance. From this it is deduced that the segregation in the F_2 will skew when plotted on an arithmetic scale, but will become normal when plotted logarithmically. Plotting the data for crosses 3313 and 3210 in such a manner demonstrates the correctness of the preceding deduction. The logarithmic curves for these two varieties are given in Fig. 2, and it will be observed that both are very similar to the normal curve. The only abnormality noticed is the erratic nature of segregation at the upper end of the range.

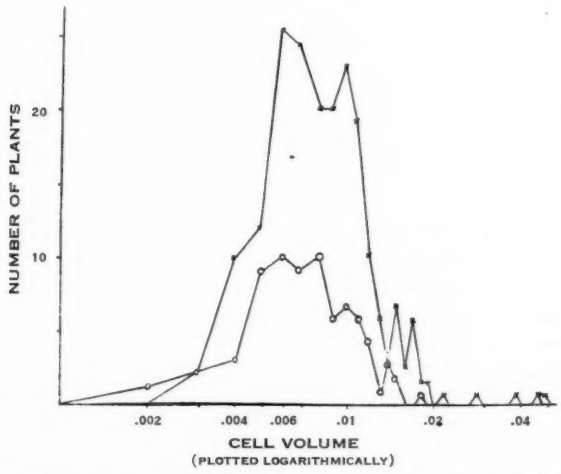

FIG. 2. *Segregation of cell volume in the F_2.* —o— *3313 F_2 (R.C. × 902 selection).* —x— *3210 F_2 (R.C. × Tangerine).*

As the parental cell sizes are not recovered in either of the crosses, the method adopted for predicting the number of segregating genes for ovary volume, cannot be used here. Other methods have been tried but none have proved satisfactory. Most of the known methods are based on the surmise that size genes have an equal effect. Such an assumption does not seem valid for cell expansion.

Many of the methods of estimating the number of genes are based on the standard deviation. There are two methods of obtaining this statistic; it may

be obtained from either the arithmetic measurements or from their logarithms. It has been the custom to use the arithmetic series for such calculations, but the asymmetry of the arithmetic curve in tomato leaves such a procedure open to grave objections. On the other hand the use of the logarithmic series for the calculation of the standard deviation is also objectionable because of the arithmetic nature of environmental variation. It is always assumed that environmental variation is arithmetic, and such an assumption is indicated in tomato by the normality of the arithmetic curves for the P_1 and F_1 generations; Kapteyn (14), however, has pointed out that the geometric is more plausible. The normality of the curves may be the result of lack of numbers, large populations being required to distinguish between the two in such cases. Since neither method is without objection an example using each method will be given and the results by the two methods will be compared.

In cross 3313 the standard deviation calculated from the arithmetic series is 0.00416, and the mean of the series is 0.00876 cu. mm. The two parental values are 0.001 cu. mm. and 0.059 cu. mm. From a table of probability integrals, the chances of obtaining the parental values and the number of plants that must be grown in order to obtain them can be worked out. The cell size of Red Currant, the smaller parent, is 2.1 standard deviations from the mean, and from a table of probability integrals it is found that cells of this size and smaller would be expected to occur twice in each hundred plants in the F_2. Turning next to the upper end of the range and to the larger parent, it is found that the value for cell size of the large parent is 12 standard deviations above the mean of the F_2. Although with the normal curve infinitely large values can occur, the chances of getting a value even six standard deviations away is 5,000,000,000 : 1, and the chances of getting a deviation equal to 12 standard deviations is so infinitely small that the value is not included in the ordinary tables.

The standard deviation of the logarithmic series of cross 3313 is 0.226 and the mean is 0.897. The two parental values are 0.000 and 1.771. The chances of obtaining the small parental size are 4 in 10,000 and the chances of obtaining the large parental size are 7 in 10,000. The chances of getting either parental size are both equal. The probability of recovering either of the parental sizes in the F_2 is quite small in samples of the size usually grown, but if an F_2 generation of 1000 plants was grown there would be an even chance of getting one plant with the cell size of one or other of the parents. These considerations lead us to prefer the standard deviation of the logarithms when working this statistic for use in estimating the number of factors involved in size crosses. In his selection experiment with mice, Goodale (9) based all his calculations on the arithmetic scale and concluded that his selected mice must have mutated in order to reach their final size. If his figures were recalculated on a geometric scale it would not be necessary to postulate any new mutations.

Discussion

Consideration of the tomato data and a critical examination of the literature causes the author to raise several objections to the simple additive scheme of inheritance. These objections are:

1. The inapplicability of the arithmetic mean to measurements of all characters of the individual. If linear measurements are studied and found to fit the arithmetic mean, then it is impossible for weight, volume, or area to fit the same arithmetic scheme since they depend upon a power of the linear measurements. An arithmetic scheme raised to any power other than one is no longer an arithmetic scheme; the interval between consecutive members of the series is now different. This fact has not been recognized by other investigators although it was pointed out by Kapteyn, in reference to the normal curve, as early as 1916. The use of the geometric mean is free from this objection as *a geometric series can be raised to any power and still remain a geometric series* with equal logarithmic intervals between successive terms.

2. The F_1 is closer to the geometric mean than it is to the arithmetic mean of the parental values. Groth (11, 12), and Dale (5), both stated that their data were fitted much better by the geometric mean than they were by the arithmetic one. Sinnott (24) found that the percentage method fitted his cucurbit data, whereas a simple additive scheme did not. MacArthur and Butler (17) gave extensive data for fruit weight in the tomato to show that the F_1 mean is the geometric mean of the parent means, and not the arithmetic mean of the parental averages. They also presented a working hypothesis of size inheritance in which they claimed the geometric mean was a more rational value than the arithmetic average. If the data in the literature are examined in the light of this hypothesis, it is found that, although not recognized by the investigator, the data fit the geometric mean, as well as, or even better than they do the arithmetic mean.

There are two main reasons why investigators have failed to notice the similarity between their results and the geometric mean. In the first place, when the differences in size between the two parents are not great, there is very little difference between the arithmetic and geometric mean; either could be applied equally well to the data. In the second place there are a number of results in which dominance of small size has been postulated; such results are usually compatible with a geometric interpretation.

As examples of the first case may be quoted the work of Sosa-Bourdouil (26) with plants, and Wexelsen (27) with animals. Sosa-Bourdouil, working with broad beans, postulated an intermediate value for the F_1, but the F_1 weight that he obtained, i.e., 0.8 gm. from a cross in which the parental weights were 1.4 and 0.49 gm., is closer to the geometric mean of 0.83 than it is to the arithmetic mean of 0.95 gm. In pigeons, Wexelsen found the F_1 beak length in a cross of Field (23.8 mm.) $\times$ Bagadette (34.0 mm.) to be 28.4 mm. The geometric mean was 28.4 and the arithmetic mean, 28.9 mm. It will be seen that although the geometric mean fitted the data better

than the arithmetic mean, there was really no significant difference between the two means. In examples of this type the arithmetic mean can be expected to fit the data just as often as the geometric does; therefore they have no diagnostic value.

As an example of dominance of small size Maw's (18) work on domestic fowl can be quoted. He said, "There is a tendency for the F_1 mean weight to approach the mean weight of the smaller parent". Examination of his figures show that they fit in well with the geometric mean. In his cross of Brahma (3350 gm.) × Sebright (708 gm.), the F_1 weight was 1612 gm. The geometric mean is 1590, and the arithmetic mean is 2129 gm. The geometric mean gives a good fit without postulating dominance, but the arithmetic mean does not.

3. The F_2 frequency curves for weight, cell size, and cell number, were all strongly skew in a positive direction when plotted in arithmetic metre, but became normal when plotted on a logarithmic scale. Such a finding has two interpretations: either gene action is proportionate and not additive or small fruit size is dominant over large fruit size. The second interpretation is not to be favoured as it assumes the dominance to be only partial and of exactly such a degree that the F_1 mean is always close to the geometric mean of the two parents. The chances of such an occurrence are remote, especially when one considers that genes controlling several processes would have to show the same type of dominance behaviour. Sinnott (24) obtained the same type of curves and postulated proportionate action of genes to explain the asymmetry.

The asymmetry of the F_2 offers a grave objection to the theory of simple additive action of size genes. The fact that the use of logarithms makes the skew curve symmetrical is one point in favour of the geometric or proportionate action of size genes.

4. The unreasonable nature of probability integrals calculated from the standard deviations of the additive scheme. The standard deviations for cell size show that there are 10,000,000,000 more chances of obtaining the small parental size than there are of obtaining the large parental size. The use of standard deviations from the logarithmic series shows these chances to be equal. The logarithmic or proportionate interpretation, therefore, appears to give more rational results.

5. The proportionate nature of physiological effects. Qualitative genes that affect fruit size were found by MacArthur and Butler to produce a constant percentage increase or decrease (17). This is compatible with the work of Robb (22), who found that the percentage growth rate is identical for Flemish giant, hybrid, and Polish rabbits from birth to the onset of puberty.

The interpretation placed on the results obtained in tomato is that the action of qualitative genes is to produce an increase or decrease proportionate to the size attained in the absence of these genes. The question therefore is raised, "If qualitative genes affect fruit size in this proportionate manner, why should typical size genes as such not do the same?"

The proportionate nature of the increase is also to be seen in linkage studies where the linkage of a quantitative trait with a qualitative character is investigated. The work of Sax (23), Sirks (25), and Rasmusson (21) shows the proportionate nature of the linkage effect, although the investigators themselves give only the absolute increase.

The geometric or proportionate nature of the size effects of known genes makes it extremely probable that size genes as such also show this proportionate effect.

These five objections make it clear that the tomato data, as well as most of the size inheritance data in the literature, are not compatible with the simple additive theory of gene action. The geometric hypothesis is open to none of these objections and it can now, it is thought, be adopted in place of the additive one.

Recently Powers (19, 20) in a study of the interactions of locule number, fruit shape, and fruit size came to the conclusion that "The effects of the genes differentiating number of locules were geometrically cumulative. Also this is true of the effects of genes differentiating fruit size". His quantitative results are in agreement with those of MacArthur and Butler and are additional evidence in favour of the geometric hypothesis of size inheritance.

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CANADIAN WILTSHIRE BACON

XIX. COMPARATIVE FLAVOUR TESTS ON CANADIAN AND DANISH BACONS[1]

BY C. A. WINKLER[2] AND W. H. COOK[3]

Abstract

Tests of the comparative flavour quality of Danish and Canadian bacon, conducted in England by jury groups and a large body of representative consumers, showed, on the average, that Danish bacon was preferred. Nevertheless the product from certain Canadian factories was of quality comparable with the best Danish bacon. This shows that the better curing practices and transport conditions already developed in Canada, in use when these tests were made, are capable of delivering to the British consumer a generally satisfactory product. The average quality of Canadian bacon would therefore be improved by the uniform adoption of these improved practices. Although toughness, texture, flavour, and fat of poor quality may all have contributed to the inferiority of Canadian bacon, excessive saltiness appeared to be the principal complaint.

Introduction

The results of several physical, chemical, and bacteriological studies on Canadian Wiltshire bacon have been presented in recent communications from these laboratories (1–4, 6–13). Although palatability, particularly with respect to flavour, was recognized as the fundamental criterion of quality, no attempt was made to examine this factor for two main reasons. *(a)* Flavours can only be judged subjectively, and to take account of consumer preference it is obviously necessary to make flavour tests with representatives of the consuming public concerned. Since practically all the Wiltshire bacon of Canadian manufacture is exported to England a reliable consumer estimate of its palatability could not be obtained except by experiments in which representative English consumers acted as judges. *(b)* Flavour tests made in Canada could, at best, be arranged to take only doubtful cognizance of possible changes in flavour during transport and subsequent smoking and distribution.

To satisfy these two main requirements for reliable information, all the flavour tests reported in this paper were made in England under the supervision of a representative of the National Research Laboratories (C.A.W.).

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Experimental Designs

Approximately 50% of the bacon consumed in England at the time of these studies was of Danish origin. Whether as a result of acquired preference by the consumers, or of intrinsic superiority of the product, Danish bacon may be regarded as a standard of Wiltshire bacon quality on the English market. Hence, the investigations were primarily designed to compare the qualities of Danish and Canadian bacons as reflected in consumer preference, especially in relation to palatability. Experiments were also designed, however, to determine the variability in product exported from various factories in Canada.

Of the Canadian bacon received in England, approximately 75% is consumed in the southern counties, particularly around London, and the remainder in the northern counties. In the south, the bacon is retailed as a smoked product, whereas in the north the major portion is consumed in the pale condition. There is, moreover, a difference in selection and cut in the two areas. Investigations were therefore made in both areas.

A. London Area

The studies in the London area were of several types.

1. *Jury Group Tests with Pale and Smoked Bacons*

To obtain information about the effect of smoking, there was designed, according to a factorial scheme, an experiment in which smoked and pale bacons from 10 Danish and 10 Canadian factories were to be provided to two jury groups of six people each. The original design called for four samples of pale bacon, representing a random selection of factories, to be tested each day by one group, and four samples of smoked bacon from the same sides as the pale materials to be tested by the second group. Tests were to be made on each set of four samples on two successive days, so that in 10 days samples from all 20 factories would have been tested in duplicate. The whole experiment was then to have been repeated, but owing to difficulties in securing materials, it was not possible to reproduce exactly, in this second half of the experiment, the arrangement of samples used in the first half. In consequence of this the data were amenable only to limited statistical analyses, and are correspondingly limited in their significance.

2. *Canteen Tests*

Smoked material from 10 Danish and 10 Canadian factories, taken at random, was provided in sample pairs, representing all possible combinations of the 20 sides to be tested, to various canteens in the London district. Patrons of the canteens served as judges. In all, 360 sample pairs were provided, 18 sample pairs being furnished each day for 20 days.

3. *Incomplete Randomized Block Tests*

Samples from 252 sides of smoked bacon, representing the product from 10 Danish and 18 Canadian factories taken at random, were tested by two entirely independent jury groups of nine people each. The samples were

combined to conform to a 7 × 9 balanced incomplete randomized block scheme, and each jury member compared four samples per day during seven days. At the end of this time, each member had tasted all the samples once. This experiment had a decided advantage over the other experiments that were made, since in it nine instead of two sides were used to represent each factory, thus affording a reliable estimate of the palatability of the product from each factory.

4. *Family Group Tests*

Simultaneously with the incomplete randomized block scheme described above, sample pairs from the same materials, in all possible combinations of Danish and Canadian bacons, were provided to 54 households in London and the surrounding provinces. Each household received a sample pair each day during seven days.

5. *Consumer Tests*

To supplement the information from the jury group and family tests, and to satisfy the desirability of having a reasonably large cross section of the population represented, sample pairs of smoked materials from a random selection of 10 Danish and 10 Canadian plants, representing all possible combinations of the 20 samples, were provided to 1330 consumers in and around London. The experiment was repeated the following week. It was not possible to duplicate exactly the factories represented in the two tests.

6. *Tests in J. Lyons & Co. Laboratories*

A limited number of tests were made by experienced tasters in the laboratories of J. Lyons & Co., Hammersmith, with smoked material from 10 Danish and 10 Canadian factories. A jury group of six people was employed, the system of scoring being that to which they were accustomed and more detailed than that used in the other experiments. This point will be considered further in a later section.

B. Northern Area

A consumer test, similar to *A5*, was made in the northern area (Liverpool, Manchester, Birmingham, Hull, Leeds). Pale bacon was used, however, and the method of cutting commonly employed in this area was adopted. Sample pairs representing all combinations of Danish and Canadian bacons from a random selection of 10 factories of each country were provided to 1140 consumers for comparison. The experiment was repeated the following week. The same Canadian, but not the same Danish factories were represented in the two experiments.

Experimental Materials and Methods

Although it would have been preferable to have taken the experimental materials from retail shops, such a procedure was impractical due to the great difficulty, if not impossibility, of ensuring that the desired materials could of certainty be obtained. The bacons were therefore obtained directly

from the wholesale distributors. The materials for Experiments *A1*, *A2*, and *A5* were smoked by one wholesale firm, those for *A3*, *A4*, and *A6* by another firm. The factories included in any given experiment were representative of those exporting regularly at the time.

In Experiments *A1* to *A4* and Experiment *A6*, 2 to 3 lb. portions of the long back immediately behind the ribs were removed for experimental materials. In Experiment *A5*, the whole of the long back was used, excluding material from within 2 to 3 in. of the salt pocket to the collar. For experiments in the northern area, the whole of the so-called "middle" from each side was used; this middle represents the bacon side from which the fore-ends and gammons are squarely cut, and includes all the streak, whereas only a small amount of streak remained on the long back used in the London area. These conditions represent accepted practice in the respective markets.

Bones were removed from all materials and samples sliced to uniform thickness on a machine. The slices, properly identified and allocated according to the experimental design, were immediately wrapped in grease-proof paper for future dispensation.

With sample pairs, the presence or absence of rind afforded the means by which the tasters identified the samples submitted to them for comparison. In each case, a record available only to the supervisor enabled subsequent identification for compilation of data. In the jury group tests, the samples were identified by their positions on the laboratory bench.

Samples for the jury groups were kept in a refrigerated room at 32° F. from the time of sampling until the time of cooking. This storage period was seldom more than 15 hr. Samples for household tests were sent through the mails, well wrapped and supported between pieces of cardboard. Dispatch was made immediately after sampling in late afternoon or evening and the samples were generally available for testing at breakfast the following morning. An instruction sheet and a score card were included in each envelope. Samples for the canteen tests and the consumer tests were delivered personally within 24 hr. of sampling, being held in a refrigerated room until time of delivery.

All samples tested by the jury groups were grilled, four samples being cooked at one time. Since the fat dripped through the grill no mingling of flavours was possible, unless it occurred by absorption of volatile substances. This doubtless was negligible. All the cooking for each jury group was done by a cook assigned to the group. The samples for one group were prepared on an electric grill, those for the other group on a gas-fired grill. Care was taken to cook the samples to about the same degree of crispness.

Cooking in the family group and large consumer tests could not, of course, be supervised. However, an instruction sheet accompanying each sample pair emphasized the necessity for cooking each sample separately in a clean frying pan. The extent to which this procedure was followed is not known,

but there is reasonable assurance that, at least in the smaller test involving 54 households, the instructions were well obeyed.

Cooking for the canteen tests was done by the staff in charge of cooking operations in the particular canteen. For the most part, these samples were grilled.

The tests carried out by Lyons & Co. were made on samples grilled by an experienced laboratory technician.

Where selection of personnel was possible, only persons thoroughly accustomed to eating Wiltshire-cured bacon were chosen. Selection was not possible, of course, in the canteen and large consumer tests.

One jury group, operating at the Smithfield Laboratory of the Food Investigation Board, Department of Scientific and Industrial Research, and designated in future as the Smithfield jury group, was composed of the officer-in-charge of the laboratory, his assistant, a university student, the proprietress of a restaurant, three members of an office staff, one of whom was a woman, the superintendent of a building, and a male employee of some 40 years' experience with a firm of bacon importers.

A second jury group, operating at the firm of Sheed-Thomson & Co., and designated hereafter as Importers' jury group, was composed entirely of members of the office staffs of firms importing bacon into England.

Little information about the personnel of the 54-family group was available. The group comprised several members of a university staff and various executives of business firms, and the more immediate friends of these two classes.

The personnel of the 12 canteen test groups for which data are available represented a variety of occupations and salary ranges. Included were the clerking staffs, assistant buyers and buyers of departmental stores, waiters, assistant chefs and chefs of hotels, the clerking staff of a social organization, assistant grocers, factory hands, and the staff of a cheese importing firm. There were both male and female tasters whose salary ranges could be distinguished as low, medium, and high. Although in some groups all salary ranges prevailed, in other groups low, low to medium, or medium to high ranges defined the limits.

The large consumer tests in both London and northern areas also represented a wide salary range, among employees of several firms through which the samples were distributed.

The jury groups compared four samples at a sitting, each member working quite independently of other members of the group. No effort was made to compel a taster to adopt any of the frequently recommended procedures such as rinsing the mouth with water, or eating a piece of apple or bread between tasting different samples. Occasionally, a water rinse was voluntarily used, particularly if a sample was distastefully salty. The majority of the tasters preferred to swallow the sample after chewing it, and this was not discouraged.

It was felt that each taster should be allowed to function in the manner considered most desirable from his or her personal point of view.

In the family group tests, the large consumer tests, and the canteen tests, no restrictions whatever were placed on the method of tasting, even to the point of permitting other foods to be eaten with the bacon in the normal way if desired. No doubt academic objections might be raised to these methods, but from a practical point of view there is probably much to commend them.

In all tests except those conducted by Lyons & Co., the taster was simply required to place the samples provided in order of preference stating, where possible, reasons for the preference.

The jury groups were required to place four samples in order of preference at each sitting; the consumer tests, family, and canteen tests involved only a distinction between one sample pair per sitting. The taster was free to state, where desired, that no distinction was possible between any two or more samples provided.

On the basis of the preference so obtained it is possible to assign numerical values. For sample pairs, these are simply $+1$ for the preferred sample, -1 for the sample not preferred and 0 for each if neither sample is preferred. With four comparisons the scores are 1.03, 0.30, -0.30, and -1.03 in decreasing order of preference (5). The intervals between successive scores were chosen to transfer the ordinal numbers obtained in scoring to normal deviates amenable to analysis of variance. If any two or more samples are indistinguishable, each is assigned the average of the scores corresponding to the two or more positions taken by the identical samples in the order of preference.

Results

1. *Jury Group Tests with Pale and Smoked Bacons (London Area)*

As mentioned previously, the original design of these experiments was factorial. Owing to circumstances over which no control could be exercised, however, the second half of the experiment could not be made a duplicate of the first half, nor could the experiments of the last day be completed.

Since samples from the same factories were tested on two successive days, comparisons between days are possible but, since the grouping of the factories within days was not the same for the duplicate tests as during the first 10 days' experiments, comparisons between sides are not possible. The procedure adopted was to treat the results from the second half of the experiment as if an additional number of factories was represented, rather than duplicate tests of material from the same factories represented in the first half of the experiment. Also, since results for the last day could not be obtained, those for the second last day were omitted. A total of 72 tests, obtained during 18 days, by each of the two jury groups remains for consideration.

Since comparisons are possible within each two-day period, the results from nine groups of four factories each may be treated independently, and the observed variance allocated as shown in Table I. The difference between

TABLE I

ANALYSIS OF VARIANCE OF JURY GROUP DATA WITHIN SUCCESSIVE TWO-DAY TEST PERIODS FOR FIRST 18 DAYS OF TEST

Variance due to:	D.f.	Mean square	
		Smithfield jury, smoked bacon	Importers' jury, unsmoked bacon
Between factories (within two-day test periods)	27	129**	89.1*
Between days (factories × days)	27	23.0*	44.0**
Residual	270	16.0	19.0

Bacon	Total score	Bacon	Total score
Pale:		Smoked:	
Danish	265.35	Danish	373.08
Canadian	−265.35	Canadian	−373.08

* *Indicates 5% level of significance.*

** *Indicates 1% level of significance.*

NOTE: *Significance of differences between factories determined by comparison with variance between days and latter compared with residual.*

days, which is the interaction of factories × days, significantly exceeds the residual variance for both jury groups, and the difference between factories has therefore to be tested against this interaction. On this basis, the difference between factories is found to be significant within two-day test periods, averaged for all days for both jury groups.

If the scores are totalled over the daily periods, 14 in all, in which two Danish and two Canadian sides were tested on each day, comparable scores by countries are obtained and these are reported in the last part of Table I.

Evidently, Danish bacon showed a superiority over Canadian in both the pale and smoked conditions. The figures also indicate that flavour quality in the pale and smoked conditions are directly related, and that inferiority in the pale condition is exaggerated by smoking. However, these indications are open to question in the absence of an estimate of the difference between sides from the same factory, against which the differences recorded above must be tested for significance.

2. *Canteen Tests (London Area)*

The results for the canteen tests are even more limited than those described above for the jury group tests on the same materials owing to commencement of hostilities in Europe, which disorganized arrangements for the return of the score cards distributed with the samples. Information for days 13 and 15 to 20 inclusive was lacking, and the results for the first 12 days only are therefore considered. Owing to the method of scoring, by preference

only, and the fact that different canteens were used each day, only a limited statistical treatment is possible.

Since two canteens tested the same samples on successive days, it is possible to analyse the variance into portions attributable to within and between canteens. The results of such an analysis are shown in Table II. Not only is the difference between canteens not significant, but the mean squares are almost identical. This demonstrates the rather interesting point that although some variation may occur between different individuals, ability to discriminate between bacon flavours did not seem to bear any relation to salary class or occupation.

TABLE II

ANALYSIS OF VARIANCE OF RESULTS OBTAINED IN THE CANTEEN TESTS

Variance due to:	D.f.	Mean square
Between canteens	36	0.85
Within canteens	144	0.81

In Table III are given the total mean scores, arranged by test periods and country of origin of the product. The results are comparable only within test periods but the totals by countries over 10 test periods confirm the results of the jury group tests in showing the Danish product to be superior.

TABLE III

TOTAL MEAN SCORES FOR SMOKED BACON FROM FACTORIES TESTED IN CANTEEN EXPERIMENTS

Tests (days)	Canadian factories	Total mean scores × 100	Danish factories	Total mean scores × 100
1–2	*C*-1	00.0	*D*-1	66.0
	C-2	−82.0	*D*-2	16.0
3–4	*C*-3	00.0	*D*-3	00.0
	C-4	−50.0	*D*-4	50.0
5–6	*C*-5	00.0	*D*-5	−16.0
	C-6	−84.0	*D*-6	100.0
7–8	*C*-7	84.0	*D*-7	00.0
	C-8	−100.0	*D*-8	16.0
9–10	*C*-9	50.0	*D*-9	−66.0
	C-10	−16.0	*D*-10	32.0
	Totals	−198.0	Totals	198.0
11–12	—	—	*D*-4	00.0
			D-6	−50.0
			D-1	66.0
			D-2	−16.0

NOTE: *Results comparable within tests (days) only. Necessary difference within tests = 103.*

3 and 4. *Incomplete Randomized Block and Family Group Experiments (London Area)*

The samples used for the jury group tests according to the randomized block scheme were taken from the same sides as the sample pairs for the family group tests, and it is convenient to examine the results of these two experiments together. Complete analyses of variance were possible on these data, and little comment is necessary.

In Table IV, the factories have been arranged in decreasing order of total score over both jury groups for each country of origin. The standard error

TABLE IV

FACTORY TOTALS OBTAINED IN JURY GROUP AND FAMILY GROUP TESTS

Factories (in order of total score for both jury groups)	Group *A* (Smithfield)	Group *B* (Importers')	Sum (*A* + *B*)	Difference (*A* − *B*)	Family group
Canadian[1]					
A-1	265	59	324	206	7
B	142	4	146	138	7
A-2	18	90	108	−72	3
C-1	156	−76	80	232	−5
D	−21	58	37	−79	−1
E	−103	139	36	−242	−5
A-3	98	−106	−8	203	4
F	37	−64	−27	102	−6
G-1	−106	62	−44	−167	−4
H	−236	184	−51	−420	1
C-2	−166	98	−68	−264	−10
C-3	−243	165	−79	−408	−1
1	−100	−106	−206	5	1
G-2	−180	−118	−299	−62	−8
G-3	−80	−260	−340	180	−1
J	−287	−120	−407	−167	−3
K	−164	−314	−478	151	−9
L	−282	−207	−489	−75	−4
Danish[1]					
M	349	120	469	229	4
N-1	251	149	401	102	5
O	269	106	375	164	5
P-1	160	120	279	40	12
Q-1	152	18	170	134	4
Q-2	−134	295	161	−429	2
P-2	8	49	57	−41	9
R-1	100	−82	18	183	5
R-2	37	−40	−3	77	−4
N-2	59	−221	−162	281	−8
Standard error of single plant total	±163	±169	±235	±235	±7.81
Necessary difference, 5% level of significance, between any two factory totals	452	468	651	651	14.69

[1] *Letters and associated numbers designate companies within nations, and factories within companies, respectively.*

of a single factory total, and the differences necessary for statistical significance between two such totals, are recorded at the bottom of the table.

The corresponding data for the total scores over the two jury groups, and for their differences, are also given, together with the data for the family group tests.

Tables V and VI give the results of variance analyses on the data of Table IV. From Table V it is evident that the Smithfield Laboratory jury group

TABLE V

ANALYSES OF VARIANCE OF FACTORY TOTALS OBTAINED IN JURY GROUP TESTS

Source of variance:	Group *A* (Smithfield)		Group *B* (Importers')		Sum (*A* + *B*)		Difference (*A* − *B*)	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Between all factories	27	458,482**	27	311,014	27	456,729**	27	312,767
Canadian vs. Danish	1	3,480,005**	1	583,453	1	3,456,656**	1	603,868
Between Canadian	17	370,487	17	305,909	17	365,790	17	310,778
Between Danish	9	288,971	9	290,387	9	295,178	9	284,180
Error	162	230,015	162	247,534	324	238,774	324	238,774
Canadian								
Between companies	11	407,841	11	245,438	11	484,817*	11	259,371
Between factories within companies	6	307,005	6	416,772	6	147,448	6	405,024
Danish								
Between companies	5	189,141	5	206,938	5	269,373	5	250,019
Between factories within companies	4	416,944	4	394,647	4	327,435	4	326,882

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

TABLE VI

ANALYSES OF VARIANCE OF FACTORY TOTALS OBTAINED IN FAMILY GROUP TESTS

Source of variance:	D.f.	Mean square
Between all factories	27	2.42**
Canadian vs. Danish	1	12.9**
Between Canadian	17	1.83
Between Danish	9	2.38*
Error	351	1.17
Canadian		
Between companies	11	2.35*
Between factories within companies	6	0.88
Danish		
Between companies	5	1.41
Between factories within companies	4	3.60*

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

was able to demonstrate a significant difference between factories, taken over all factories regardless of origin, and a highly significant difference between Danish and Canadian bacons. The data from the Importers' group, although subject to only a slightly larger mean square error, did not demonstrate any source of variance to be statistically significant. However, the results based on the totals over both jury groups show the differences, not only between factories and between Danish and Canadian bacons, but also the difference between Canadian companies, to be significant. Hence, the individual results of the two groups must be supplementary.

An analysis of variance of the difference between the two jury groups shows none of the sources of variance recognized in the tables to be statistically significant. Since this analysis really represents the different reactions of the two groups to the quantities reported in the table, it is evident that one group did not prefer one, and the second group another type of bacon.

Variance analyses on the results of the family group test, Table VI, give support to the conclusions reached from the jury group tests. Significant differences between factories, taken over all factories, and between Danish and Canadian bacons were again demonstrated. Three other sources of variance were, however, shown to be significant, namely, between Danish factories over all Danish factories, between Canadian companies, and between Danish factories, within companies. For Canadian bacon, therefore, the major source of variance lies between companies, whereas for Danish bacon it lies within companies. The inference would seem to be that, whereas differences in flavour in Canadian bacons from different factories arise primarily from differences in curing and handling practices, variations in flavour of Danish bacons are presumably due to variations between pigs, or possibly to slight time to time variations in handling practices in a given factory.

Table VII summarizes the comments of the members of the jury groups. In summarizing these comments, only two classes were distinguished, namely, favourable and unfavourable. Unfavourable comments include those in which a sample was said to be very salty, salty, or slightly salty; if no comment was made on the saltiness, a favourable rating for this quality factor was assigned to the sample. If no comment whatever was made on a sample no rating was assigned, whereas if, for example, a taster reported the sample as both tough and salty, a double entry was made. All the attributes of quality mentioned by the tasters are included in the table. Since there were 18 Canadian factories and only 10 Danish factories, the summary is expressed on a percentage basis.

The comments summarized in this form show that, taken over all the recognized attributes of quality, both jury groups agreed that Canadian bacon is inferior to Danish, a fact that merely bears out the flavour scores. The remarks indicate that both Danish and Canadian bacons were considered too salty for the English palate, with no marked inferiority of the Canadian product in this respect; on the other hand, Canadian bacon suffered considerably more adverse criticism for toughness than did Danish bacon. A

TABLE VII

SUMMARY OF COMMENTS MADE BY JURY GROUP PANELS (SMITHFIELD AND IMPORTERS')

Quality factors	Favourable[1]		Unfavourable[1]		Unfavourable, as % of total	
	Group *A* (Smithfield)	Group *B* (Importers')	Group *A*	Group *B*	Group *A*	Group *B*
Canadian factories (18)						
Lean						
Saltiness	4	2	63	77	94.0	97.5
Toughness	18	20	15	29	45.5	59.2
Texture	3	19	4	32	57.2	62.8
Flavour	22	73	30	18	57.7	19.8
Miscellaneous	14	26	14	1	50.0	37.5
Fat	17	2	5	10	22.7	83.2
Total	78	142	131	167	63.9	54.1
Danish factories (10)						
Lean						
Saltiness	2	3	22	26	91.8	89.7
Toughness	12	17	5	7	29.4	29.2
Texture	0	11	1	12	—	51.1
Flavour	18	40	10	17	35.7	29.8
Miscellaneous	10	16	1	0	9.1	—
Fat	7	1	2	5	22.2	83.2
Total	50	88	41	67	45.0	43.2

[1] *The ratings are based on comments for individual samples compared in blocks of four samples per test.*

general conclusion regarding flavour, considered as a quality factor in itself, is scarcely possible, and the same is true for texture. The fats of the two types were more frequently criticized for off-flavour by the Importers' group, but there is little to choose between the fats as determining quality in the two bacons.

It is a point of considerable interest and importance that although Canadian bacon as a whole was regarded as inferior to Danish, the total scores recorded in Table IV show that it is not impossible for Canadian factories to turn out a product closely approaching the best Danish product in quality. A major problem remains, however, in bringing the quality of Canadian bacon generally to a higher level.

5. *Consumer Tests (London and Northern Areas)*

Although, as mentioned previously, 2660 sample pairs were distributed in the London area, and 2240 pairs in the northern counties, approximately 50% of the people in the tests failed to submit an opinion about the samples. Normally, the organization of the experiments would have been adequate to ensure returns in the neighbourhood of at least 90%, but international crises, culminating in war, made it impossible to supervise return of the score cards.

Owing to the incomplete returns, statistical analysis of the data is necessarily limited. The experimental error, pooled over both series, was computed for each of the London and northern areas, from the available number of degrees of freedom. The necessary difference for a given comparison was computed from these values. Since the score from each factory in a given series is represented by a *total*, the necessary difference appropriate to such a score is given by:

$$(\text{Mean square for individual comparison} \times n)^{\frac{1}{2}} \times t$$

The necessary difference therefore increases with the number of comparisons available for each factory, and since the number of returns per factory varies,

TABLE VIII

CONSUMER TESTS OF SMOKED BACON IN LONDON AREA

Factories (in order of score over both series)	Series I			Series II			Mean score for both series
	Number of factories in comparison	Number of comparisons between pairs	Total[2] score	Number of factories in comparison	Number of comparisons between pairs	Total[2] score	
Canadian							
10 each series							
*C–A*1	19	58	− 1	14	41	15	7.0
*C–B*1	18	65	12	11	26	0	6.0
*C–C*1	18	73	15[1]	14	50	− 9[1]	3.0
*C–D*1	17	62	− 3	15	43	0	− 1.5
*C–A*2	19	66	−15	19	75	4	− 5.5
*C–E*1	—	—	—	18	54	− 7	− 7.0
*C–F*1	—	—	—	16	50	− 9	− 9.0
*C–B*2	19	72	−10	14	50	− 9	− 9.5
*C–G*1	—	—	—	16	58	−10	10.0
*C–H*1	19	77	−11	—	—	00	−11.0
*C–I*1	19	74	−22[1]	15	40	−10[1]	−16.0
C–12	19	77	−21	—	—	—	−21.0
C–13	19	76	−23	—	—	—	−23.0
Danish							
10 each series							
D–1	19	70	17	—	—	—	17.0
D–2	19	66	15	—	—	—	15.0
D–3	—	—	—	18	45	13	13.0
D–4	18	78	16	16	41	2	9.0
D–5	—	—	—	14	34	8	8.0
D–6	18	62	3	16	44	9	6.0
D–7	19	63	16[1]	15	46	− 6[1]	5.0
D–8	18	75	5	14	44	5	5.0
D–9	18	65	5	—	—	—	5.0
D–10	—	—	—	13	39	4	4.0
D–11	—	—	—	16	52	3	3.0
D–12	18	59	5	16	48	0	2.0
D–13	18	67	2	14	50	− 3	− 0.5
D–14	19	79	− 5	—	—	—	− 5.0

[1] *Factories in which there was significant difference between series.*

[2] *Within countries and series, the following differences in score, or greater, are statistically significant between plants:*

Canadian, Series I: 24. *Canadian, Series II:* 19.
Danish, Series I: 17. *Danish, Series II:* 20.

the necessary difference for the score is different for almost every comparison that might be desired.

In the London area (Table VIII), individual comparisons between series, within factories, show that three factories differed significantly between series. In the northern area (Table IX), no comparisons for the Danish factories are possible, owing to the impossibility of having the same factories represented

TABLE IX

CONSUMER TESTS OF PALE BACON IN NORTHERN AREA

Factories (in order of score over both series)	Series I			Series II				Mean score for both series
	Number of factories in comparison	Number of comparisons between pairs	Total score	Number of factories in comparison		Number of comparisons between pairs	Total score	
Canadian								
10 each series								
*C–A*1	18	51	1[1]	19		64	24[1]	12.5
*C–B*1	18	64	9	15		59	4	6.5
*C–C*1	17	56	15[1]	16		64	−17[1]	− 1.0
*C–D*1	19	73	− 2	16		64	0	− 1.0
*C–E*1	17	38	− 2	16		64	− 1	− 1.5
*C–F*1	15	47	1	11		64	− 6	− 2.5
*C–G*1	19	70	− 4	15		68	− 4	− 4.0
*C–D*2	19	68	− 7	14		58	− 2	− 4.5
*C–B*2	18	60	2[1]	16		65	−17[1]	− 7.5
*C–E*2	19	73	− 4	12		48	−17	−10.5
Danish								
10 each series								
D–1	17	63	17	*D*–11	17	71	10	—
D–2	16	58	7	*D*–12	16	58	15	—
D–3	19	68	− 2	*D*–13	15	61	9	—
D–4	18	68	0	*D*–14	13	47	7	—
D–5	18	65	− 1	*D*–15	15	57	5	—
D–6	18	64	11	*D*–16	17	70	− 7	—
D–7	19	69	−11	*D*–17	13	53	3	—
D–8	15	49	−11	*D*–18	14	61	3	—
D–9	19	69	3	*D*–19	14	51	−11	—
D–10	18	65	−22	*D*–20	18	67	2	—

[1] *Indicates factories showing significant difference between series within countries and series; differences in score of 19 or greater indicate statistically significant differences between factories.*

in the two series. The results from three Canadian factories, however, showed significant differences between the two series. Several of the insignificant differences are large enough to suggest that, had the regular analysis of variance been possible, the difference between sides within factories (between series) would have been a significant source of variance. It appears from this that the results from two sides are not representative of the product from a given factory. If this is so, more complete returns probably would not have enabled more definite conclusions. The experimental error likely would have remained the same, but a significant difference between sides within factories

would probably have been the basis for comparing the differences between factories.

In view of the possible significant difference between series (between sides from the same factories), but lacking a suitable test of significance, each series has been treated separately. The difference between factories within series is therefore compared with the necessary difference arising from experimental error. Again, each pair of factories has a unique necessary difference, depending on the total number of tests for which returns were made. Such comparisons, although valid for the particular sides tested, may not be valid with respect to factories, owing to the limited number of sides used to represent each establishment. For this reason, and also to simplify and reduce the results, only the largest necessary difference for comparisons within a given nation and series are reported at the foot of each table.

The results in Table VIII show that even on this basis significant differences do exist between factories.

In Table X the total scores are given by countries and series, together with the necessary differences for determining whether they are significantly above or below the average (zero) and whether the total differences between scores are significant. The table is practically self-explanatory. In three out of the four tests, the difference between the scores for Danish and Canadian bacons are statistically significant. In one test (Series I, London area) Danish bacon was found significantly better than average.

TABLE X

CONSUMER TESTS OF PALE AND SMOKED CANADIAN AND DANISH BACONS

Area	Series	Scores			Necessary difference		
		Total for Canadian	Total for Danish	Total	From average (0) Canadian	From average (0) Danish	Between totals for countries
Smoked							
(London) *L*1	1	−79	79	0	44.8	44.3	71.2
(London) *L*2	2	−35	35	0	37.1	35.5	51.3
Pale							
(Northern) *N*1	1	9	9	0	39.3	40.5	56.4
(Northern) *N*2	2	−36	36	0	40.4	39.6	56.6

The fact deserves special comment that, whereas in the London area both series show a significant superiority of Danish over Canadian bacon, the tests in the northern area showed Danish superior to Canadian bacon in only the second series. The difference in the results from the two areas might be explained by the generally accepted belief that the people in the northern counties are less discriminating than those in the London area. However, it should be borne in mind that, whereas in the London area both types of

bacon are smoked shortly after arrival in England, similar measures of a preservative nature are not practised in the northern area. The apparently smaller superiority of Danish over Canadian bacon in the northern tests might therefore reflect a better keeping quality of the Canadian product in the pale condition.

Tables XI and XII summarize the available comments of tasters in the London and northern areas respectively. Only about 50% of the tasters commented on the samples, so that the numbers appearing in these tables do not correspond with those in the previous tables.

TABLE XI

SUMMARY OF COMMENTS MADE BY TASTERS ON SMOKED BACON; CONSUMER TESTS, LONDON AREA, SERIES I AND II

Quality factor	Favourable[1]	Unfavourable[1]	Unfavourable, as % of total
Canadian factories			
Saltiness	33	31	48
Toughness	10	12	55
Flavour (unspecified)	187	241	56
Miscellaneous	15	18	55
Total	245	302	55
Danish factories			
Saltiness	35	37	51
Toughness	14	12	46
Flavour (unspecified)	222	168	35
Miscellaneous	21	18	46
Total	292	235	45

[1] *Ratings based on comments from 537 comparisons out of 1157 sets of paired samples on which flavour scores were obtained.*

TABLE XII

SUMMARY OF COMMENTS MADE BY TASTERS ON PALE BACON; CONSUMER TESTS, NORTHERN AREA, SERIES I AND II

Quality factor	Favourable[1]	Unfavourable[1]	Unfavourable, as % of total
Canadian factories (10)			
Saltiness	56	54	49
Toughness	6	7	54
Flavour (unspecified)	205	226	52
Miscellaneous	61	70	53
Total	328	357	52
Danish factories (10)			
Saltiness	52	54	51
Toughness	10	9	47
Flavour (unspecified)	232	211	48
Miscellaneous	80	71	47
Total	374	345	48

[1] *Ratings based on comments from 702 comparisons out of 1226 sets of paired samples on which flavour scores were obtained.*

From the comments, the attributes of quality listed in the tables were recognized. The miscellaneous comments include such factors as colour, amount of fat, etc. Where the product received a favourable comment it was given a score of 1 and the sample with which it was in comparison a score of −1. The number of unfavourable comments is expressed as a percentage of the total, to take into account the variation in number of comments involving each type of bacon.

On the whole, the rating given by the comments reflects essentially the same results as the flavour scores, namely that superior samples were more frequently obtained from Danish bacon. There is no evidence that Canadian bacon was considered more salty than the Danish product, nor, apparently, was the toughness of Canadian bacon detrimental to an appreciable extent. In the unspecified flavour comments, however, smoked Danish bacon assumes a considerable margin of superiority in the London area, though this is not true for the tests in the northern area.

6. *Tests in J. Lyons & Co. Laboratories (London Area)*

Although very limited in scope, the tests made in the laboratories of J. Lyons & Co. are of considerable interest in view of the detailed system of scoring employed. Numerical values were assigned to each distinguishable characteristic of both lean and fat. For the lean, these characteristics are colour, taste, tenderness, juiciness, and flavour; for the fat, only taste, flavour, and texture were evaluated. Such a system of scoring has obvious advantages over the simpler preference rating used in the experiments already outlined, but if reliable results are to be obtained, requires operators, such as those in the Lyons' group, who are experienced in its use.

To obtain the data from the Lyons' group in a form suitable for statistical treatment, certain of the results had to be omitted. One member of the group was frequently absent, and the results from this individual were discarded completely, leaving five instead of six members represented. Results for one day had also to be disregarded, owing to substitution for one of the jury members. The results finally represent comparisons between eight Danish and nine Canadian factories.

The mean scores for each attribute for each factory and country are shown in Table XIII, arranged in decreasing order of total score. Necessary differences applicable to the means by factories and countries have been calculated and appear at the foot of the table. For certain attributes of quality some factories appear to differ significantly when compared on the basis of these necessary differences. However, an analysis of variance shows that for some of these attributes no significant difference exists in fact. In such cases the values have been put in parentheses. The apparent conflict arises from the fact that a 5% point computed from differences selected at random, is not the maximum difference to be expected when a number of comparisons are made. In these circumstances the variance analysis is the correct basis for assessing significance.

TABLE XIII

MEAN SCORES FOR EACH ATTRIBUTE OF QUALITY FLAVOUR TEST DATA, LYONS' JURY GROUP

Factories and countries	Total over all attributes of quality	Scores							
		Lean					Fat		
		Colour	Taste	Tenderness	Juiciness	Flavour	Flavour	Taste	Texture
Possible score	100	15	10	15	10	15	15	10	10
Canadian									
C–1	84.0	13.6	8.3	13.2	8.5	12.2	11.9	7.9	8.4
C–2	83.4	12.2	7.6	12.7	8.1	11.7	13.7	8.8	8.6
C–3	82.0	12.1	8.4	11.5	7.6	12.0	13.0	8.8	8.6
C–4	80.6	12.8	7.3	12.2	7.7	10.4	13.5	8.5	8.2
C–5	80.0	11.6	8.2	11.8	7.5	11.4	12.6	8.7	8.2
C–6	79.9	11.8	7.5	12.0	7.5	11.8	12.5	8.6	8.2
C–7	78.3	12.9	7.6	10.9	8.1	10.4	11.9	8.5	8.0
C–8	75.2	11.0	6.7	11.5	7.5	10.7	12.4	7.9	7.5
C–9	74.7	9.7	6.6	11.9	7.9	11.0	11.9	8.1	7.6
Mean	79.79	11.97	7.58	11.97	7.82	11.29	12.60	8.42	8.14
Danish									
D–1	87.9	13.2	8.2	13.3	8.5	12.5	14.0	9.1	9.1
D–2	86.0	12.1	8.8	12.9	8.1	12.7	13.5	9.0	8.9
D–3	85.5	12.1	8.4	13.3	8.4	12.3	13.3	9.1	8.6
D–4	83.5	11.7	8.3	13.8	8.5	13.0	12.3	7.9	8.0
D–5	83.4	13.3	7.6	12.2	8.1	11.4	13.3	8.8	8.7
D–6	82.5	11.7	8.5	12.8	8.0	11.1	12.9	9.0	8.5
D–7	81.9	11.0	8.0	13.6	8.1	11.8	13.0	8.3	8.1
D–8	80.9	13.0	7.7	12.5	8.2	11.0	11.7	8.4	8.4
Mean	83.95	12.26	8.19	13.05	8.24	11.98	13.00	8.70	8.54
Necessary difference									
Between countries	1.90	(0.75)	(0.81)	0.68	0.34	0.47	(0.46)	(0.34)	0.24
Between factories	5.36	(2.20)	(2.85)	(1.98)	(0.98)	1.37	(1.34)	(1.00)	0.71

NOTE: *Values in parentheses failed to reach level of statistical significance.*

TABLE XIV

RESULTS OF ANALYSES OF VARIANCE ON THE FLAVOUR TEST DATA OBTAINED BY THE LYONS' JURY GROUP

Source of variance	D.f.	Mean square							
		Total of all attributes of quality	Lean				Fat		
			Colour	Taste	Tender-ness	Juici-ness	Flavour	Flavour	Taste	Texture
Between all factories	16	123**	9.81	3.82	6.74	1.25	6.14*	5.00*	1.74	1.78*
Canadian vs. Danish	1	733**	3.70	15.7	49.7**	7.30*	19.9**	6.78	3.26	6.54**
Between Canadian	8	107*	13.8	4.25	4.65	1.25	4.74	4.63	1.32	1.53*
Between Danish	7	54.7	6.83	1.64	3.00	0.40	5.76	5.17	2.00	1.40
Error (factories×days)	16	34.0	5.37	6.14	3.05	0.85	3.04	1.54	0.93	0.38

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

The main interest in the present investigation is the comparison of different factories and countries of origin. A detailed analysis of variance established the fact that the interaction "factories × days" was an adequate estimate of error against which to test the significance of differences between countries of origin and between factories. The variance analysis given in Table XIV shows that for the totals of all attributes of quality the differences are significant between factories, over all factories, between Canadian and Danish bacon, and between Canadian factories, but are not significant between Danish factories. Tenderness, juiciness, and flavour of the lean and texture of the fat are apparently the principal factors contributing to the difference between countries of origin of the bacons. Comparison of the mean values of Table XIII with the significant differences demonstrated by the variance analysis shows that the Danish product is superior in respect of the corresponding attributes of quality. In general, the results from the Lyons' jury group are in agreement with those from the experiments discussed previously but have the advantage that the reasons for the Danish superiority can be somewhat more clearly defined.

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THE STUDY OF THE EFFECT OF TEMPERATURE UPON PUPATION[1]

By Geoffrey Beall[2]

Abstract

The problem investigated is that of dealing with the cumulative effect of temperature on the progress of the pupation of an insect. There is suggested a statistical model that appears to correspond with physical reality. On the basis of this model, there is suggested an analysis whereby one may determine the effect of temperature upon pupation when insects have been exposed to known varying temperatures and the total period required for pupation is known. The analysis, when applied to data obtained under regularly oscillated temperatures, furnishes a relationship fitting the data.

1. Introductory

Although it is well known that the time spent by an insect in pupation is dependent on the temperature to which the pupa is exposed, the establishment of empirical relationships has been hampered by the peculiar situation that only the aggregate effect of many temperatures, which is known by the duration of pupation, can be observed and, generally, not the effect of a given temperature for a given period. In much experimental work the complexities of the situation have been avoided, to some extent, by dealing with constant temperatures and considering the results per unit period; however, it appears that even this simple situation demands some consideration. We propose to suggest such a quantitative model of the development as will be amenable to statistical test and furnish a basis for the analysis of data obtained under varying temperature.

2. The Measurement of Development

The study of development with respect to temperature has been deeply influenced by the work of Shelford (6) who dealt with the velocity at which a process such as pupation goes forward, velocity being defined as the reciprocal of the time taken to complete the process under constant temperature. Rather than velocity we prefer the concept of the fraction of development or reorganization of a pupa that occurs in a unit period. The two concepts are close and have indeed been used interchangeably, as by Ludwig and Cable (4). In order to visualize reorganization as a sum of additive parts, reorganization, although an intangible quantity, should, as a varying condition, be distinguishable from one time to another and, under constant temperature, be referable to a scale of time; hence stages of the reorganization should correspond to fractions of the total time required for pupation. Specifically, if at the

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constant temperature, t_i, the j^{th} pupation occupies n_j unit periods, then in any such period it completes, of its reorganization, a fraction,

$$\varphi_j = 1/n_j \tag{1}$$

Our idea is to refer the progress of pupation to a scale of time just as in educational research the intangible quantity, intelligence, is referred to age.

In carrying out an investigation on development under constant temperature, it is natural to treat a group of pupae, rather than one; assuming that a lot of L_i pupae are exposed to the constant temperature, t_i, and that the $j^{th}(j = 1 \ldots L_i)$ completes its pupation in n_{ij} unit periods and in one period a fraction, as in Equation (1),

$$\varphi_{ij} = 1/n_{ij} \tag{2}$$

The end of such experimental work as that under discussion is the estimation of the expectation, φ_i, of φ_{ij}, for all possible values of j, customarily made as

$$f'_i = 1/n_{i.} \tag{3}$$

where

$$n_{i.} = 1/L_i \sum_j n_{ij} \tag{4}$$

is the mean period required for pupation in the sample. It is obvious, however, that the estimate of Equation (3) may be replaced by a more direct estimate of φ_i, viz.,

$$f_i = (1/L_i) \sum_j 1/n_{ij} \tag{5}$$

Since, unfortunately, we lack data on individual pupae we cannot determine any differences, $f'_i - f_i$. There is reason, however, to suppose that such differences will vary in magnitude inversely with the magnitude of φ_i, so that if calculation follow Equation (3), the form of the relationship to varying t_i will be affected.

If the concept of a fraction of reorganization, or indeed that of velocity of development, is to be fruitful there must be a certain simplicity in the course of development with time as follows: in pn_{ij} $(0 \leqslant p \leqslant 1)$ unit periods the j^{th} pupa will reach a definite, perhaps recognizable, point in its reorganization and it must be supposed that always the j'^{th} pupa kept at the constant temperature, t_i', and requiring a total period, $n_i'_j'$, will attain the same state of reorganization in $pn_i'_j'$ unit periods. Thus we must suppose that the departure from expectation, $\varphi_{ij} - \varphi_i$, in the fraction of development completed in a unit period by a given pupa is characteristic of the whole period of its development. Such a concept is equivalent to saying that among the i^{th} group of pupae, subjected to the temperature, t_i,

$$\varphi_{ij} = k_{ij}\varphi_i \tag{6}$$

where k_{ij} is a constant peculiar to the j^{th} pupa. From Equation (6) it follows that $\sigma_{\varphi_{ij}}$, the standard deviation of the rate of development over all possible values of j and under a given temperature, t_i, is φ_i of $\sigma_{k_{ij}}$, the standard deviation of a constant peculiar to the j^{th} pupa and independent of the given temperature; thus it has been effectively supposed that $\sigma_{\varphi_{ij}}$ should be pro-

portional to φ_i. We cannot, however, test this point, and hence our primary assumption, directly by an examination of estimates of φ_{ij} because we lack data on individual pupae. From information available, however, on the period of pupation we can determine whether Equation (6) is substantially true. It is well known that in a case such as the present where n_{ij} is by definition the

TABLE I

THE TIME* REQUIRED FOR PUPATION BY MALE *Drosophila melanogaster*, WHEN SUBJECTED TO CONSTANT TEMPERATURE, AN ESTIMATE OF $\sigma_{n_{ij}}$ AND THE APPROXIMATE VALUE OF φ_i.

Temperature, °C.	Number of flies emerging	Pupation in days	$s_{n_{ij}}$	f'_i
15.0	40	14.15 ± 0.036	0.34	0.07
20.0	92	6.78 ± .008	.11	.15
22.5	63	5.27 ± .012	.14	.19
25.0	118	4.26 ± .007	.11	.23
26.0	35	4.00 ± .006	.05	.25
27.0	108	3.78 ± .003	.05	.26
27.5	110	3.65 ± .002	.03	.27
28.0	71	3.56 ± .005	.06	.28
28.5	47	3.45 ± .004	.04	.29
29.0	104	3.40 ± .004	.06	.29
29.5	105	3.35 ± .004	.06	.30
30.0	66	3.35 ± .003	.04	.30
31.0	21	3.35 ± .008	.05	.30
32.0	24	3.50 ± .008	.06	.29
33.0	17	3.63 ± .016	.10	.28

* *After Ludwig and Cable (4).*

reciprocal of φ_{ij}, with an expectation of φ_i, the standard deviation, $\sigma_{n_{ij}}$, of the period, n_{ij}, of pupation, will be, to a first approximation $1/\varphi_i^2$ that of φ_{ij}. Consequently, if $\sigma_{\varphi_{ij}}$ is proportional to φ_i, then $\sigma_{n_{ij}}$ must vary approximately inversely as φ_i. Estimates of $\sigma_{n_{ij}}$ (actually the estimated probable error of $n_{i.}$) are given by Ludwig and Cable (4) who reported the performance under constant temperatures of *Drosophila melanogaster* Meigen; their results for males are included in Table I, where, from Equation (3) we have presented f'_i as an estimate of φ_i and $s_{n_{ij}}$ as an estimate of $\sigma_{n_{ij}}$. It can be seen that there is a suggestion that the two estimates vary inversely so that our theory may be true.

3. The Simple Relationship between Development and Variable Temperatures

Generally, the end of work with a number of groups of pupae kept under various constant temperatures, is to obtain estimates of the quantities, φ_i, that may be plotted against the temperature, t_i. For any one group, however, the problem is that of estimating central tendency. Now, however, we wish to deal in a similar way with the development as it occurs under varying temperatures, such as occur naturally; then the problem becomes one of

determining directly a function of temperature. For the moment we shall consider a simple relationship between variable temperature and development in the sense that we shall attempt to explain the effect of variable temperatures as simply an aggregate of the effects of a number of constant temperatures. To a large extent, such a simple relationship is adequate although variability also clearly has other effects, as is discussed in Section 5.

Suppose as in Section 2, that the j^{th} pupation is completed in n_j periods, and that the j^{th} pupa, at the temperature, t_s, of the s^{th} period effects, by analogy with Equation (1), a fraction, φ_{js}, of its total development. When the temperature is not constant, it can be said that

$$\sum_s \varphi_{js} = 1 \tag{7}$$

where the summation is made over the n_j periods occupied by the j^{th} pupation. Equation (7), in which it is known only that the development that occurs in all the periods under the conditions peculiar to each finally results in the completion of pupation, illustrates our initial point, in Section 1, that the effect of temperature can only be seen in the aggregate.

It is necessary to make an estimate of a general fraction, φ_s, of development in the s^{th} period, such that, on the substitution of φ_s for φ_{js}, Equation (7) will be satisfied as nearly as possible for all values of j. Let us assume that there may be obtained an estimate, F_s, of φ_s, which when substituted for φ_{js} in Equation (7) will yield a discrepancy,

$$\xi_j = 1 - \sum_s F_{js} \tag{8}$$

and that F_{js} may be chosen so that $\sum_j \xi_j^2$ is minimal. Now as indicated above, φ_s must be a function of t_s. Guided by general experience with the quantities, φ_i, it may be assumed that φ_s does not vary abruptly with changes in temperature and, further, that

$$\varphi_s = a_0 + a_1 t_s + a_2 t_s^2 + a_3 t_s^3 + \ldots . \tag{9}$$

where a_0, a_1 etc. are constants. From Equation (9) the minimization of $\sum_j \xi_j^2$ will be straightforward. It will be necessary ultimately to gain some experience with respect to the nature of the variability of the quantities with which we are dealing when perhaps some other form of estimation will be found more suitable.

4. The Effect of Regularly Oscillating Temperatures

In investigations on the effect of temperature upon the course of pupation there has been a natural tendency to study the effect of oscillating temperatures as compared with the effect of a temperature that is the mid-point of the oscillations. The problem and its possible solution are illustrated below with the data of Mikulski (5). He kept 14 groups of pupae of *Tribolium confusum* Duval at various temperatures and recorded the mean time spent in pupation by each group as is shown in our Table III. Let us deal with time in half-days, since Mikulski varied the temperature at such intervals, and assume that the i^{th} group of pupae completed pupation in n_i half-days

for unfortunately, these records must be treated as if the mean result for a given group of pupae obtained for a single, i^{th}, pupa; this is not strictly correct when applying the foregoing discussion.

We shall not proceed to elaborate the general solution for variable temperatures as indicated in Section 3 because of a modification necessitated by Mikulski's presentation. First, note that he restricted his treatments to subjection of the pupae either to a constant temperature, or to two alternating temperatures, e.g., for the i^{th} group, these temperatures were $T \pm R$, and that each group of pupae was distinguished by a peculiar pair of values of T and R. Write as before, the fraction of development completed ideally in a half-day at the temperature, $T \pm R$, as $\varphi_{T\pm R}$ with, as in Equation (9),

$$\varphi_{T\pm R} = a_0 + a_1(\mathrm{T} \pm R) + a_2(T \pm R)^2 + a_3(T \pm R)^3 + \ldots \qquad (10)$$

Then for an entire day, assume that the fraction of development was

$$\begin{aligned} \varphi_{TR} &= \varphi_{T+R} + \varphi_{T-R} \\ &= 2a_0 + 2a_1T + 2a_2(T^2 + R^2) + 2a_3(T^3 + 3TR^2) + \ldots \end{aligned} \qquad (11)$$

From Mikulski's discussion of his experimental procedure it is not clear with which temperature, of a given pair, he commenced so that we cannot distinguish between the two temperatures and must suppose that the pupae were exposed equally to each temperature, as must have been approximately the case. Since development was completed in $n_{i.}/2$ days, we can write, as in Equation (7),

$$n_{i.}\varphi_{TR}/2 = 1 \qquad (12)$$

Then, as was discussed in connection with Equation (8) we can make an estimate, F_{TR}, of φ_{TR} by getting from Equation (11) and Equation (12), by the method of least squares, for all observed values of i:

$$\left.\begin{aligned} a_0\Sigma_i n_{i.}^2 + a_1\Sigma_i n_{i.}^2\, T + a_2\Sigma_i n_{i.}^2\,(T^2 + R^2) + \ldots\ldots\ldots &= \Sigma_i n_{i.} \\ a_0\Sigma_i n_{i.}^2\, T + a_1\Sigma_i n_{i.}^2\, T^2 + \ldots\ldots\ldots\ldots\ldots\ldots &= \Sigma_i n_{i.}\, T \\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots & \end{aligned}\right] \qquad (13)$$

and solving for a_0, a_1 etc.

Let us consider the number of constants that will be necessary in estimating φ_{TR} for the present data. Since inspection of Mikulski's results suggests that $\varphi_{TR}]_{R=0}$ will probably tend to approach the T-axis gradually, as T diminishes, and tend to maximize for the greatest values of T used in the experimental work, we may suppose that a third order equation, with four constants, will represent Equation (10) and hence Equation (11) in a satisfactory manner. As a test, we compared the sum of the squared deviations about the relationship, F_{TR}, when fitted with four constants (10 degrees of freedom), i.e., 0.0668, with the corresponding figure, 0.0635 obtained from five constants; from this work it was apparent that the third order was adequate. The estimates of the constants, a_0, a_1 etc., for Equation (11), of course, apply equally to Equation (10) and we choose to present them in the

latter connection, writing that generally for any temperature, $T \pm R$, acting for a half-day, the fraction of development was

$$F_{T\pm R} = +0.313,886 - 0.042,944,3\,(T \pm R) + 0.001,896,969\,(T \pm R)^2 - 0.000,024,134,5\,(T \pm R)^3 \qquad (14)$$

For the range of temperatures, in degrees Centigrade, covered by Mikulski, the relationship, Equation (14), is shown in Table II and in Fig. 1, where it can be seen that the relationship is sigmoid as was anticipated.

TABLE II

THE FUNCTION OF EQUATION (14) TABLED FOR THE RANGE OF THE EXPERIMENTAL WORK

$T \pm R$	18	19	20	21	22	23	24	25	26
$F_{T\pm R}$	.015	.017	.021	.025	.030	.036	.042	.049	.055
$T \pm R$	27	28	29	30	31	32	33	34	35
$F_{T\pm R}$	.062	.069	.075	.081	.087	.091	.095	.098	.100

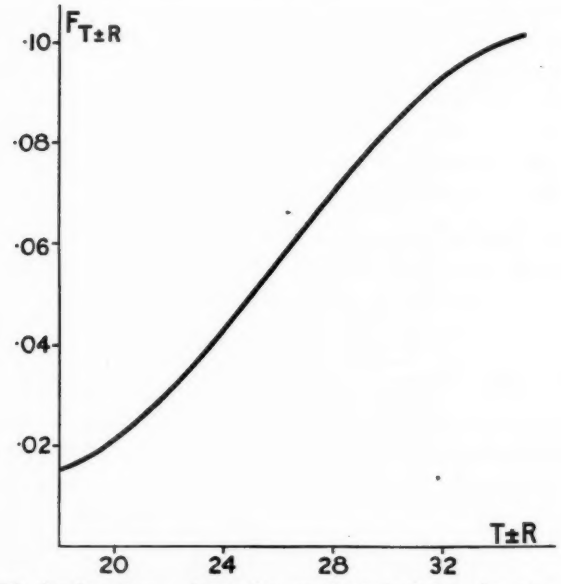

FIG. 1. *The fraction, $F_{T\pm R}$, of pupation completed in half a day at a temperature, $T \pm R$, as in Equation (14) and Table II.*

As a verification of our work, we obtained, by using the constants from Equation (14) in Equation (11), estimates of the fraction, F_{TR}, of the development that should be completed in one day under the conditions of Mikulski's experiment and contrasted this quantity with the fraction observed to have been completed, i.e., $f'_{TR} = 1/n_{i.}$, as shown in Table III, where it can be seen that a fair correspondence exists.

TABLE III

THE CALCULATED AND OBSERVED FRACTIONS OF DEVELOPMENT COMPLETED IN ONE DAY WITH THE VARIOUS COMBINATIONS OF ALTERNATING TEMPERATURE USED BY MIKULSKI

$T-R$	22.5	20.0	17.5	25.0	22.5	20.0	17.5	27.5	25.0	22.5	20.0	30.0	27.5	25.0
$T+R$	22.5	25.0	27.5	25.0	27.5	30.0	32.5	27.5	30.0	32.5	35.0	30.0	32.5	35.0
n_i.	14.40	13.67	13.32	9.90	11.88	9.34	8.90	6.70	8.08	8.30	8.30	5.90	6.06	6.90
f'_{TR}	.069	.073	.075	.101	.084	.107	.112	.149	.124	.120	.120	.169	.165	.145
F_{TR}	.066	.069	.080	.098	.099	.102	.107	.131	.130	.126	.121	.162	.159	.149

5. The Effect of Variability in Temperature

Mikulski was interested particularly in the effect of variability in temperature on development. In our terms, Mikulski's discussion on the difference between the rate of development under oscillating temperatures and the rate under a constant temperature (the mid-point of the oscillations) can be expressed as an investigation for a given value of T, on

$$d_{TR} = f'_{TR}]_{R\neq 0} - f'_{TR}]_{R=0} \tag{15}$$

To d_{TR} we may contrast, for a given value of T, the quantity,

$$\begin{aligned} D_{TR} &= F_{TR}]_{R\neq 0} - F_{TR}]_{R=0} \\ &= (+0.003{,}793{,}94 - 0.000{,}144{,}807\,T)R^2 \end{aligned} \tag{16}$$

by substitution of the values of a_0, a_1 etc., as in Equation (14) in Equation (11). In Equation (16), of course, $D_{TR} = 0$ when $R = 0$ and when $R \neq 0$, $D_{TR} < 0$ for $T > 26.2$ and $D_{TR} > 0$ for $T < 26.2$. These inequalities are essentially the conclusions on which Mikulski remarked when he showed that pupae subjected to two temperatures, $T \pm R$, did not complete their development in the same time as those subjected to the constant temperature, T. The present work suggests that the effects that he detected could be largely explained by saying that the mean of a curvilinear function at any two values of the independent variable must generally differ from the value of the function at the mid-point of the independent variable. It is probable that to a large extent the problem of the effect of variable temperatures that vexes the ecological literature could be resolved in terms of a simple curvilinear relationship, as in Fig. 1, between the fraction of development, completed in a unit period, and temperature.

Inasmuch as evidence of a directly experimental nature has generally shown that the response of an organism to a given temperature is conditioned by its previous experience of temperatures, there undoubtedly exist some effects that cannot be explained in terms of our simple relationship. A particularly clear case of a true effect of varying temperatures is furnished in a related field by Buchanan (3) who showed that the development of embryos of *Amblystoma punctatum* under oscillating temperatures depended clearly on the period of the oscillations. Since there is no room in terms of our simple relationship of Section 3, for the period of oscillation to have any effect, variability clearly has a proper effect. In the investigation, however, of

such true effects of variability, allowance should clearly be made first for the curvilinearity of the simple effect.

In order to establish an empirical relationship for an effect truly ascribable, in the sense just discussed, to variability in the temperature, one might perhaps, instead of saying, as in Equation (9), that development is $f(t_s)$, consider it as $f(t_s , t_{s-1})$, where t_{s-1} is the temperature of the period preceding the s^{th}. A relationship of the type suggested was fitted by Beall (1) for the activity of moths of *Pyrausta nubilalis* Hubn. and it was found that the influence of the preceding temperature was important in modifying the response to the temperature of a given period.

6. Summary and Discussion

We have attempted to build a model of the development of a pupa as a sum of additive parts gauged by time and there is some evidence in the literature that this model is adequate, although more data are required on the statistical nature of development. Incidentally, it appears that in experiments with constant temperatures, the development per unit period should be estimated by using an harmonic mean rather than the arithmetic mean usually employed. On the basis of our model, it should be possible to determine development as a function of temperature when one knows only the total time taken by pupation under variable temperature. We have suggested that such a function may be fitted by the method of least squares and illustrated the fitting on the special case of oscillating temperatures. In this case, the difference in the effect of oscillating temperatures, from that of a constant temperature (the mid-point of the oscillations) can be ascribed to curvilinearity of the response to temperature.

The present theory should not be restricted to the problems of pupation, since in connection with the duration of a larval stage or the development of an egg there must arise the same problems. Further, similar problems may arise in, for example, studying the relationship between the growth of fish and temperature when it is only convenient to measure the fish at long intervals, whereas temperature can be easily determined at intervals as short as desired. The methods suggested in the present paper may be an aid in analysing general or fundamental studies and of direct use in establishing an empirical relationship between development and environmental conditions when animals are being reared in great numbers so that a nice adjustment of conditions would be profitable.

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